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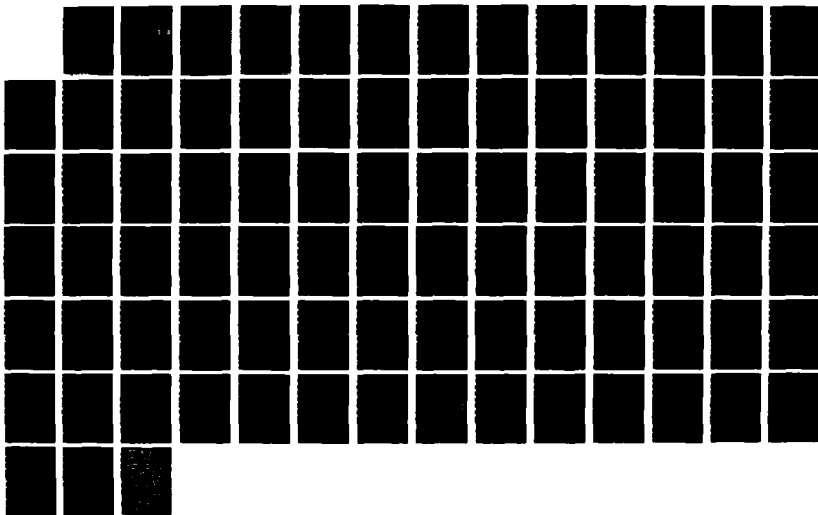
CHEMICAL PREPARATION LABORATORY FOR IND CANDIDATE
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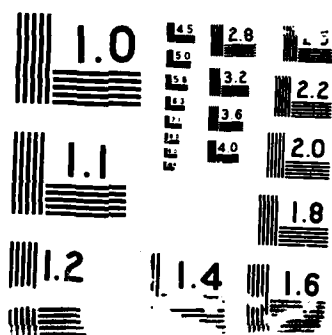
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Chemical Preparation Laboratory for IND Candidate Compounds

Annual Report

E.M. Schubert, Ph.D.

February 5, 1988

(January 17, 1987 - January 16, 1988)

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U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
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07	05				
06	03				
19. ABSTRACT (Continue on reverse if necessary and identify by block number) During the reporting period sixteen compounds were synthesized and submitted for testing. These compounds can be considered modified nucleosides derived from ribavirin, arabinoadenosine and deazaguanosine, while the other half of the compounds are based on phenanthridone as the skeletal structure. Compounds which remain under investigation are selenazole, ribavirin metabolites, triazole carboxamides and prodrug ester. A large scale preparation of ribavirin amidine hydrochloride is presently in the preparatory stage. <i>Revised 10/14/88</i>					
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I. SUMMARY

During the reporting period twenty-two target compounds have been examined, and the preparation of fourteen compounds was completed. Three of these compounds, ARA ADA, AVS 206, and AVS 360 TH were resynthesized and submitted twice.

The following compounds were delivered: Ribavirin amidine hydrochloride (AVS 206; ribavirin methylamdate (AVS 206 AE), acetylarabinoadenine (ARA ADA); combretastatin (AVS 353); combretastatin acetate (AVS 353 AC); 3-deazaguanine (AVS 272); 3-deazaguanosine (AVS 215); thiazofurin nitrile (AVS TFN), and phenanthridone derivatives AVS 360 INT; AVS 360 MA, AVS 360 TH, AVS 360 TA.

The syntheses of the following target compounds remains under investigation, and their preparations are in progress: 5-Hydroxy-1,2,3-triazole-4-carboxamide (AVS 94); 5-Hydroxy-1-(β -D-ribofuranosyl)-1,2,3-triazole-4-carboxamide (AVS 136); 1,2,4-triazole-3-carboxylic acid; 1,2,4-triazole-3-carboxamide; 1- β -D-ribofuranosyl-1,2,4-triazole-3-carboxylate; phenanthridone derivatives (AVS 360), prodrug ester and a large-scale preparation of ribavirin amidine hydrochloride (AVS 206).

II. FOREWORD

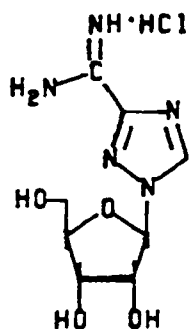
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All target compounds reported herein were prepared in strict compliance with "Current Good Manufacturing Procedures" (CGMP) guidelines. All intermediates and final products unreported in the chemical literature were fully characterized by elemental and spectral analyses.

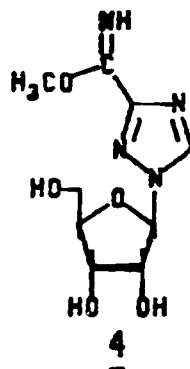
IIIa. CUMULATIVE LIST OF COMPOUNDS COMPLETED AND DELIVERED TO U.S. ARMY
MEDICAL RESEARCH INSTITUTE OF INFECTIOUS DISEASES (USAMRIID)
JANUARY 17, 1987 TO JANUARY 16, 1988

<u>No.</u>	<u>Compound</u>	<u>Amount</u>	<u>Production Control No.</u>
AVS 206	1- β -D-Ribofuranosyl-1,2,4-triazole-3-carboxamidine hydrochloride	70.0 g	1892
AVS 206	1- β -D-Ribofuranosyl-1,2,4-triazole-3-carboxamidine hydrochloride	410.4 g	1996
AVS 206 AE	1- β -D-Ribofuranosyl-1,2,4-triazole-3-methylamidate	1.15 g	2050
ARA ADA	9-(2',3'-Di-O-acetyl- β -D-ribofuranosyl)adenine	25.5 g	2011
ARA ADA	9-(2',3'-Di-O-acetyl- β -D-ribofuranosyl)adenine	23.0 g	2090
AVS 353	Combretastatin	9.5 g	1714
AVS 353 AC	Combretastatin acetate	1.0 g	1723
AVS 272	3-Deazaguanine	25.3 g	1706
AVS 215	3-Deazaguanosine	24.0 g	1873
AVS TFN	Thiazofurin nitrile	380 mg	-
AVS INT	4aH-r,1H-trans,1H-Hydroxy-1,9-methylenedioxy-1,4,4a,10b-tetrahydro-6(5H)phenanthridone	220 mg	-
AVS 360 MA	4aH-r,1H-trans,2H-cis,3H-trans,4H-trans,10bH-trans-1,3,4-Trihydroxy-2-acetoxy-8,9-methylenedioxy-1,2,3,4,4a,10b-hexahydro-6(5H)phenanthridone	1.23 g	1763
AVS 360 TH	4aH-r,1H-trans,2H-cis,3H-trans,4H-trans,10bH-trans-1,2,3,4-Tetrahydroxy-8,9-methylenedioxy-1,2,3,4,4a,10b-hexahydro-6(5H)phenanthridone	1.5 g	1746
AVS 360 TH	4aH-r,1H-trans,2H-cis,3H-trans,4H-trans,10bH-trans-1,2,3,4-Tetrahydroxy-8,9-methylenedioxy-1,2,3,4,4a,10b-hexahydro-6(5H)phenanthridone	4.4 g	2080
AVS 360 TA	4aH-r,1H-trans,2H-cis,3H-trans,4H-trans,10bH-trans,1,2,3,4-Tetraacetoxy-8,9-methylenedioxy-1,2,3,4,4a,10b-hexahydro-6(5H)phenanthridone	1.84 g	1807

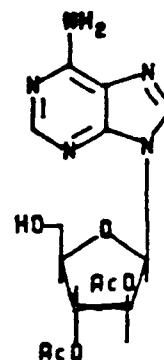
IIIb. STRUCTURES OF COMPOUNDS SUBMITTED



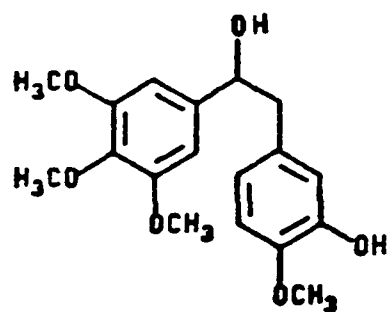
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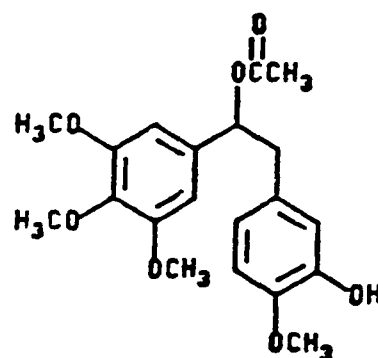
AVS-206 AE



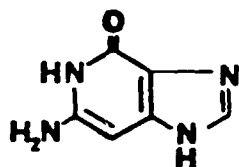
ARA-ADA



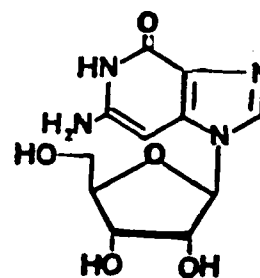
AVS353



AVS353AC

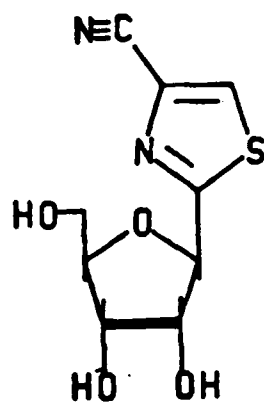


AVS-272

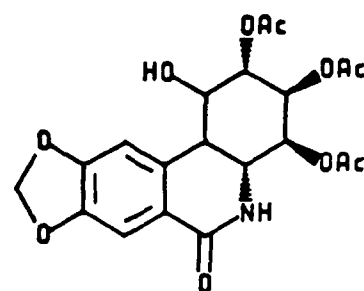


AVS 215

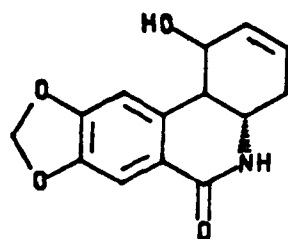
STRUCTURES OF COMPOUNDS SUBMITTED



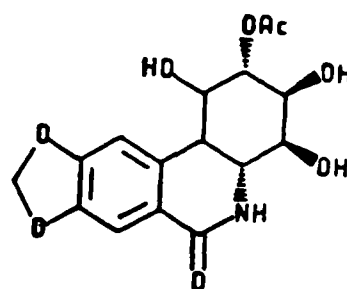
AVS-TFN



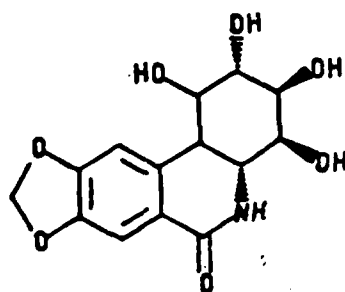
AVS3600H



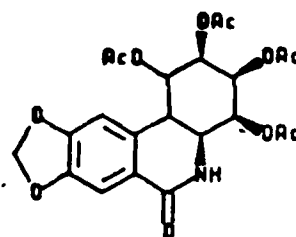
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AVS360MA



AVS360TH

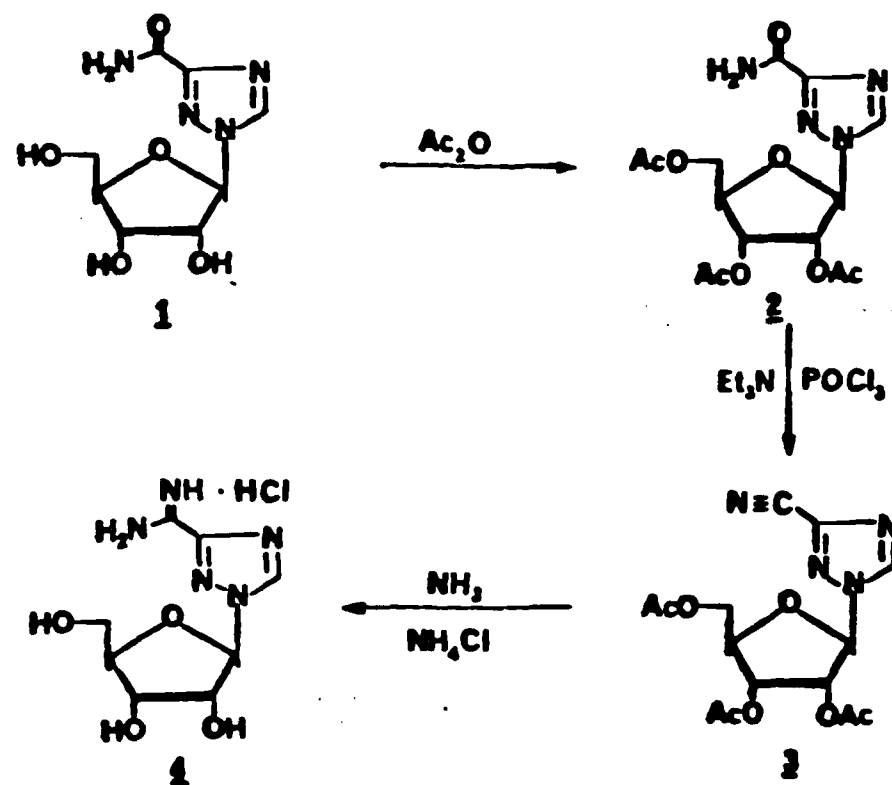


AVS360TA

IV. PROCEDURES FOR TARGET COMPOUNDS DELIVERED TO USAMRIID
from January 17, 1987 to January 16, 1988

A. 1- β -D-Ribofuranosyl-1,2,4-Triazole-3-carboxamide hydrochloride, AVS 206

AVS 206 was synthesized according to the following scheme:



Experimental¹

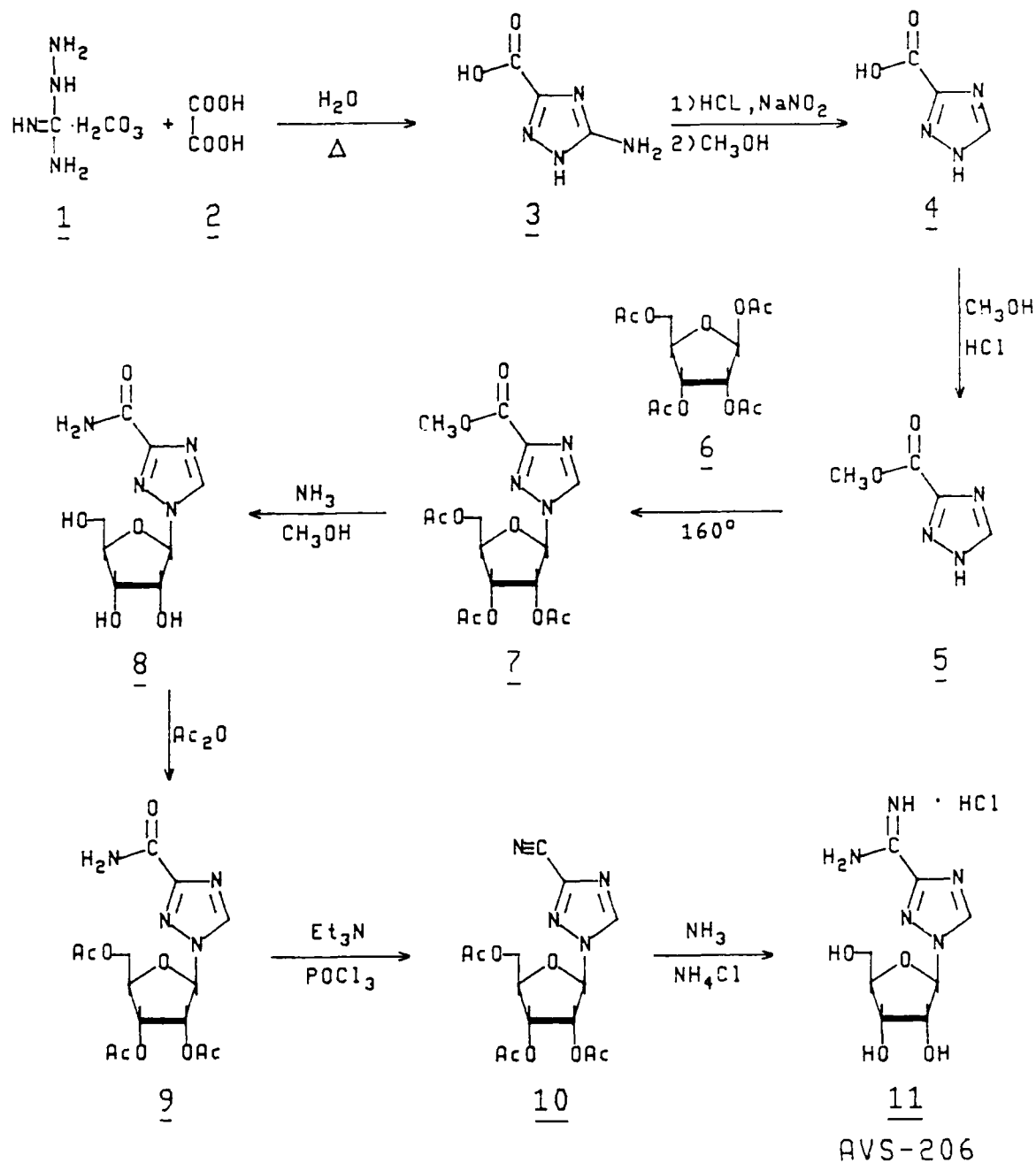
2'3'5'-Tri-O-acetylribavirin (2): A mixture of ribavirin (50 g, 0.2 mol), acetic anhydride (600 mL), and 4,4-dimethylaminopyridine (1 g) was stirred for 60 hours. Unreacted acetic anhydride was evaporated under reduced pressure at 40°. The obtained viscous residue was treated with ethanol (500 mL), and upon evaporation of the solvent the product was dissolved in cold water. The aqueous phase was extracted with ethyl acetate (3 x 300 mL). The combined organic layer was dried over sodium sulfate, and evaporated to give a solid foam which showed as a single spot on TLC. Yield: 75.8 g (100%).

1-(2,3,5-Tri-O-acetyl-β-D-ribofuranosyl)-1,2,4-triazole-3-carbonitrile (3): Tri-O-acetylribavirin 2 (74 g, 0.2 mol) and triethylamine (411 mL, 2.9 mol) were dissolved in chloroform (1200 mL) and cooled to 0°. Phosphorous oxychloride (52 mL, 0.55 mol) was added dropwise over a period of 30 minutes. The reaction mixture was kept at 0° for another 30 minutes, then the ice bath was removed and the reaction was stirred at room temperature for 3 hours. The solvent was evaporated under reduced pressure, then the residue was dissolved in ethyl acetate (1500 mL). The ethyl acetate solution was washed with water (2 x 500 mL) and with saturated sodium bicarbonate solution. The aqueous washings were combined and extracted with ethyl acetate (200 mL), the combined organic layers were dried over sodium sulfate, and treated with charcoal at room temperature. After filtering through a Celite bed the filtrate was evaporated to dryness to yield a colored syrup. The syrup was dissolved in dichloromethane (300 mL) then the solution was passed through a short column packed with silica gel, and eluted with ethyl acetate/dichloromethane 4:1 (500 mL). After evaporation of the solvent a white, crystalline material was obtained which was homogeneous on TLC. Yield: 48.2 g (68%) m.p. 96-98°; lit. 96-97°.

1-β-D-Ribofuranosyl-1,2,4-triazole-3-carboxamidine hydrochloride (4)¹: A mixture of the cyanotriazole derivative 3 (47.6 g, 0.135 mol), ammonium chloride (7.3 g, 0.137 mol) and anhydrous liquid ammonia (1000 mL) was kept in a sealed steel bomb at 90° for eighteen hours. Subsequently, the ammonia was allowed to evaporate during a twelve hour period. The residue was dissolved in ethanol (1600 mL), treated with charcoal, and filtered through a Celite bed. The ethanolic solution was concentrated to about 400 mL when the product started to crystallize. After filtration the mother liquor was concentrated to about 150 mL when a second crop was obtained. The first and second precipitate were found to be identical and they were combined, washed with ethanol and ether (100 mL) to give a total yield of 29.3 g (78%) m.p. 179-180°; lit. 177-179°.

B. 1-β-D-Ribofuranosyl-1,2,4-Triazole-3-carboxamide hydrochloride, AVS 206

AVS 206 was synthesized according to the following scheme:



Experimental^{2,3,4}

5-Amino-1,2,4-triazole-3-carboxylic acid¹ (3): Oxalic acid (11.2 Kg, 123.6 mol) was dissolved in water (255 L). While stirring aminoguanidine bicarbonate (10.5 Kg, 77.14 mol) is added portionwise. The reaction mixture is heated to 85°, and kept at 85-92° for eight hours. Upon cooling to about 70° a solution of sodium hydroxide (13.25 Kg, 50% in 10 L water) is added, then the reaction batch is reheated and kept at reflux for 90 minutes. After cooling overnight the turbid solution is filtered. The filtrate is neutralized with hydrochloric acid (4.5 L) and the resulting precipitate is collected by filtration. The product is dried at 55° while under aspirator vacuum (3 days). The product is used in the next step without further purification.

Yield: 8.81 Kg (89%); m.p. 243° (lit. 242-244°)

1,2,4-Triazole-3-carboxylic acid² (4): 5-Amino-1,2,4-triazole-3-carboxylic acid (1.4 Kg, 10.92 mol) is dissolved in hot hydrochloric acid (3.4 L conc. HCl and 8.4 L water). After cooling to 5°C a sodium nitrite solution (1.160 Kg sodium nitrite in 2.6 L water) is slowly added while maintaining the temperature below 10° by cooling in an ice bath. The precipitated diazo salt is collected by filtration and the filter cake is pressed dry without letting it go to complete dryness.

Caution: the dry diazocompound is explosive and it detonates violently when submitted to heat or friction.

A small amount of the moist diazonium salt is added to methanol (6 L at 35°), and upon initiation of the decomposition reaction strong cooling in an ice bath was required while maintaining the reaction in balance by adding small amounts of the diazonium salt. Upon completion the precipitated deamination product is collected by filtration and dried. Yield: 394 g m.p. 125-126°. This product is pure enough to be used in the subsequent esterification step.

Methyl-1,2,4-triazole-3-carboxylate² (5): To a suspension of 1,2,4-triazole-3-carboxylic acid (2.125 Kg, 18.77 mol) in methanol (12 L) hydrochloric acid gas is injected while maintaining the temperature below 20° with cooling. After gas saturation the obtained solution is left at room temperature for five days. After that time the precipitated hydrochloride salt is collected by filtration, and dried, then added to water (4 L) for hydrolysis. The methyl ester is filtered and dried. The crude material is recrystallized from boiling water (8 L) to give purified crystalline methyl-1,2,4-triazole-3-carboxylate.

Total Yield: 1247 g m.p. 198-199° (lit. 198°)

Methyl-1-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)-1,2,4-triazole-3-carboxylate² (7): Methyl-1,2,4-triazole-3-carboxylate (127 g, 1 mol) and 1,2,3,5-tetra-O-acetyl-β-D-ribose (318 g, 1 mol) are mixed thoroughly and placed in a three-neck flask equipped with a mechanical stirrer, thermometer, and take-off condenser. The flask is immersed in an oil bath preheated to 165°. After the sugar derivative has melted bis-p-nitrophenylphosphate (2.5 g) is added. After stirring for 5 minutes the pressure in the reaction apparatus is reduced and the generated acetic acid distills off. After 30 minutes the oil bath is removed, and the reaction mixture is left to cool to 50-60°.

The highly viscous, dark reaction mass is slowly poured into cold methanol (1.2 L) while stirring, and the product starts to crystallize.

Seven such fusion reactions are performed, and the combined batches are washed with methanol to give a total of 2.2 Kg (yield 61%) of methyl-1-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)-1,2,4-triazole-3-carboxylate. M.P. 104-105° (lit. 107-109°). Thin layer chromatography indicates that the product is almost exclusively the β-isomer.

1-β-D-Ribofuranosyl-1,2,4-triazole-3-carboxamide (Ribavirin)³ (8): Methanol (22 L) is placed in a stainless steel reactor and cooled to -10° by circulating alcohol at -40° through the reactor jacket. Liquid ammonia (4 L) is added to the cold methanol and the powdered acetylnucleoside 7 (2.24 Kg) is added in small portions. Upon completion of the addition the vessel is sealed air-tight, and the content is allowed to warm up to room temperature. After two days the vessel is slowly vented and the contents is heated to 35-40°, then filtered through a filter pad. The filtrate is concentrated under reduced pressure, and the precipitated solid is collected by filtration to yield 1130 g of crude ribavirin.

From the first and second mother liquor more product is obtained. Upon recrystallization from hot methanol-water 3:1 (13 L) a total yield of 1283 g (88%) is obtained. m.p. 166-167° (lit. 166-168°).

Elemental analysis, thin layer chromatography, and spectral characteristics confirm that the obtained product is the pure β-isomer of 1-D-ribofuranosyl-1,2,4-triazole-3-carboxamide (Ribavirin).

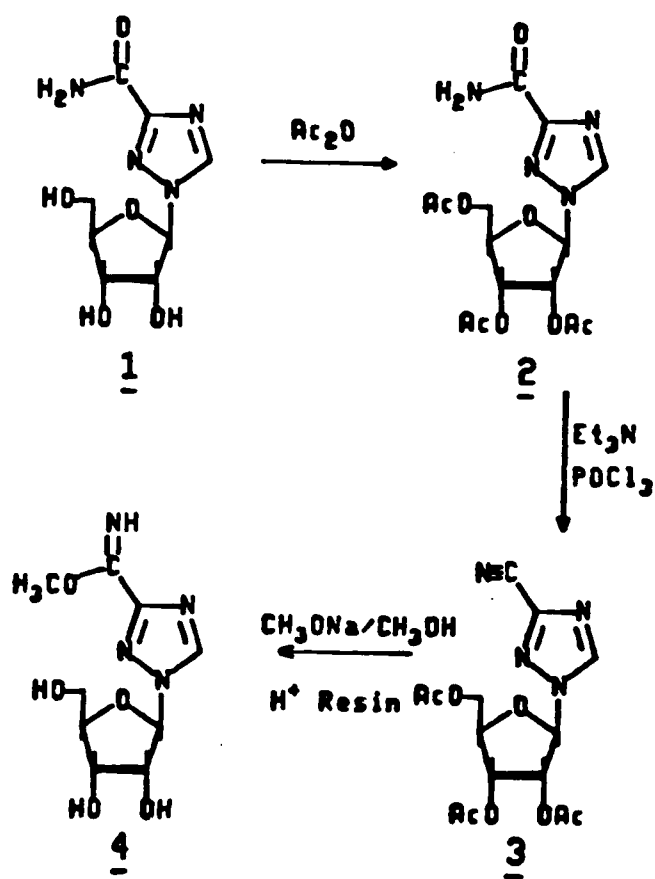
1-(2,3,5-Tri-O-acetyl-β-D-ribofuranosyl)-1,2,4-triazole-3-carbonitrile (10)⁴: A mixture of ribavirin (700 g, 2.8 mol), acetic anhydride (6 L), and 4,4-dimethylaminopyridine (14 g) is stirred for 60 hours. Unreacted acetic anhydride is evaporated under reduced pressure at 40°. The obtained viscous residue is treated with ethanol (500 mL), and upon evaporation of the solvent the product is dissolved in cold water (5 L). The aqueous phase is extracted with dichloromethane (3 x 2 L) and after drying, more dichloromethane (4 L) and triethylamine (5.7 L, 20.6 mol) are added to the tri-O-acetylribavirin solution. After cooling the solution to 5° phosphorous oxychloride (730 mL, 7.72 mol) is added dropwise over a 50 minute period, maintaining the temperature below 8°. The mixture is allowed to warm to room temperature (1 hour) with additional stirring for 2½ hours.

Ice water (5 L) and additional dichloromethane (5 L) are added, the layers are separated, and the organic layer is washed with water (2 L), dilute acetic acid (200 mL glacial acetic acid in 1800 mL water), and bicarbonate solution (100 g in 2 L water) to obtain pH 6 of the aqueous phase. The organic layer is dried, evaporated and the syrupy residue is dissolved in warm methanol (700 mL). The crystals that separate are collected by filtration to yield 500 g of the nitrile compound. The mother liquor is subjected to column chromatography on silica gel, first with dichloromethane, then with dichloromethane/acetone 4:1 as the eluant. After using the solvent mixture (25 L) another crop (130 g) is obtained upon evaporation of the solvent. Yield 624 g m.p. 95-97°. Lit 96-97°.

1-β-D-Ribofuranosyl-1,2,4-triazole-3-carboxamidine hydrochloride (11)⁴: A mixture of the cyanotriazole derivative 10 (124 g, 0.35 mol), ammonium chloride (19.1 g, 0.35 mol) and anhydrous liquid ammonia (1.2 L) are kept in a sealed steel bomb at 80-90° for 24 hours. Upon cooling the ammonia is allowed to evaporate during a 12 hour period. The oily residue is dissolved in methanol (1.9 L), heated to reflux while treated with charcoal, and filtered through a Celite bed. After cooling to 0° some material crystallizes, yielding 52.4 g of pure end product upon filtration. The mother liquor is concentrated to 700 mL, cooled, and a second crop is obtained (23.0 g). Further concentration of the mother liquor to 200 mL produces a third crop (7.5 g) which is combined with other impure material for further purification. Combining several individual crops followed by recrystallization produces a total of 412 g (85%) of analytically pure carboxamide hydrochloride 11.

C. 1- β -D-Ribofuranosyl-1,2,4-triazole-3-methylamidata, AVS 206 AE

AVS 206 AE was synthesized according to the following scheme:



AVS-206 AE

Experimental

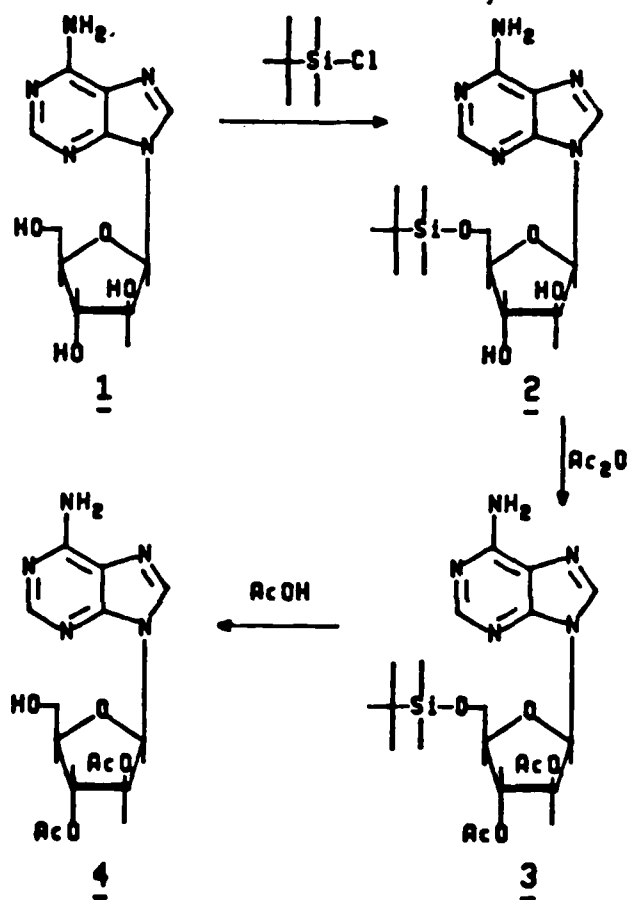
1-(2,3,5-Tri-O-acetyl- β -D-ribofuranosyl)-1,2,4-triazole-3-carbonitrile (3): A mixture of ribavirin (50 g, 0.2 mol), acetic anhydride (600 mL), and 4,4-dimethylaminopyridine (1 g) is stirred for 60 hours. Unreacted acetic anhydride is evaporated under reduced pressure at 40°. The obtained viscous residue is treated with ethanol (500 mL), and upon evaporation of the solvent the product is dissolved in cold water. The aqueous phase is extracted with ethyl acetate (3 x 300 mL). The combined organic layer is dried over sodium sulfate, and evaporated to give a solid foam which shows as a single spot on TLC.

Without further purification the obtained acetyl ribavirin (74 g, 0.2 mol) is combined with triethylamine (411 mL, 2.9 mol) and dissolved in chloroform (1200 mL) while cooling the solution to 0°. Phosphorous oxychloride (52 mL, 0.55 mol) is added dropwise over a period of 30 minutes. The reaction mixture is kept at 0° for another 30 minutes, then the ice bath is removed and the reaction is stirred at room temperature for 3 hours. The solvent is evaporated under reduced pressure, then the residue is dissolved in ethyl acetate (1500 mL). The ethyl acetate solution is washed with water (2 x 500 mL) and with saturated sodium bicarbonate solution. The aqueous washings are combined and extracted with ethyl acetate (200 mL), the combined organic layers are dried over sodium sulfate, and treated with charcoal at room temperature. After filtering through a Celite bed the filtrate is evaporated to dryness to yield a colored syrup. The syrup is dissolved in dichloromethane (300 mL) then the solution is passed through a short column packed with silica gel, and eluted with ethyl acetate/dichloromethane 4:1 (500 mL). After evaporation of the solvent a white, crystalline material is obtained which is homogeneous on TLC. Yield: 48.2 g (68%) m.p. 96-98°; lit. 96-97°.

1- β -D-Ribofuranosyl-1,2,4-triazole-3-methylamidate (4): Cyano compound 3 (3.5 g, 10 mmol) is dissolved in methanol (50 mL), then sodium methoxide (50 mg) is added to adjust the pH to 9.5. The reaction mixture is stirred for 45 minutes, then it is neutralized with ion exchange resin H⁺- form. After filtration the solvent is evaporated under reduced pressure, and the residue is recrystallized from methanol/ether to yield the pure imino ether 4; yield 1.4 g (54%), m.p. 141-142°.

D. 9-(2',3'-Di-O-acetyl- β -D-arabinofuranosyl)adenine, ARA ADA

ARA ADA was synthesized according to the following scheme:



ARA-ADA

Experimental⁵

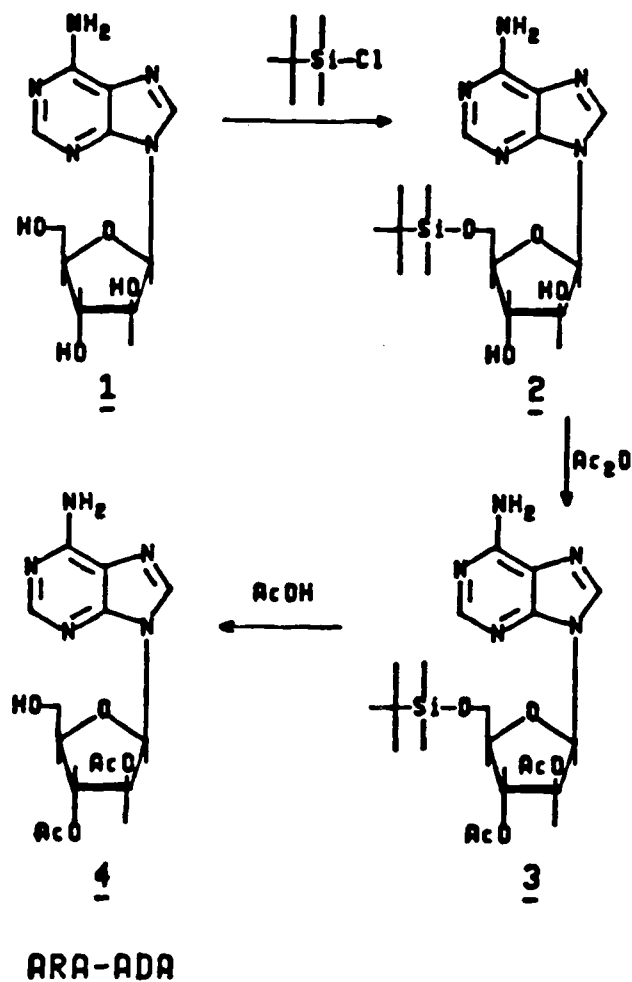
9-[5'-O-(t-Butyldimethylsilyl)- β -D-arabinofuranosyl]-adenine (2): To a stirred suspension of dry 9- β -D-arabinofuranosyl adenine (26.7 g, 0.1 mol) in dimethyl formamide (500 mL) is added imidazole (16.3 g, 0.24 mol), followed by the addition of t-butyldimethylsilyl chloride (18.1 g, 0.12 mol). The reaction mixture is stirred for 20 hours maintaining anhydrous conditions, then the solvent is removed by distillation under reduced pressure. The residue is treated with ethyl acetate (500 mL); the organic layer is washed with water, dried and evaporated. The obtained syrup is dissolved in chloroform (240 mL), the solution is warmed and hexane (200 mL) is added to give a turbid solution. After cooling to room temperature the obtained crystals are collected by filtration to give 33 g of 2. m.p. 157-158°.

9-(2',3'-Di-O-acetyl- β -D-arabinofuranosyl)adenine (4): Acetic anhydride (12.7 mL, 0.125 mol) is added dropwise to a stirred suspension of 9-[5'-O-(t-butyldimethylsilyl)- β -D-arabinofuranosyl]adenine (22.8 g, 0.06 mol) in pyridine (300 mL) at 0°. The mixture is stirred an additional 48 hours, when TLC indicates completion. Excess acetic anhydride is deactivated by adding a few milliliters of water, the solvent is evaporated under reduced pressure and the obtained syrup is dissolved in dichloromethane (500 mL). The organic layer is washed with water (2 x 100 mL), saturated sodium bicarbonate solution (100 mL) and water (100 mL). After drying over sodium sulfate the solvent is evaporated to leave 25.5 g of 3 as a solid foam. The solid is dissolved in a mixture of acetic acid (250 mL), tetrahydrofuran (100 mL) and water (100 mL) at 40° and stirred for 48 hours to remove the t-butyldimethylsilyl group. The solvent is evaporated and the resulting crude product is purified on a silica gel column, using dichloromethane-methanol 20:1 as the eluant. After evaporating the fractions containing diacetylarabino adenine the obtained solid is recrystallized from acetone to yield 10.5 g (43%) of pure product 4. m.p. 171-173° (literature m.p. 138-139° for product that contained 0.2 mol water of crystallization).

Remark: Special care has to be taken during preparation and recrystallization of 2',3'-diacetylarabino adenine, since the compound readily rearranges to form the 5'-acetyl derivative by migration of the acetyl group attached to the 2' or 3' secondary alcoholic group to give the thermodynamically favored 5' primary alcohol esterification. Such migration can be greatly minimized by working in acetic acid as the medium during removal of the 5'-silyl group. Attempts are being made to recover expensive Ara-A as the starting material by deblocking the side product, 3',5'-diacetyl Ara-A.

E. 9-(2',3'-Di-O-acetyl-β-D-arabinofuranosyl)adenine, ARA ADA

ARA ADA was synthesized according to the following scheme:



Experimental

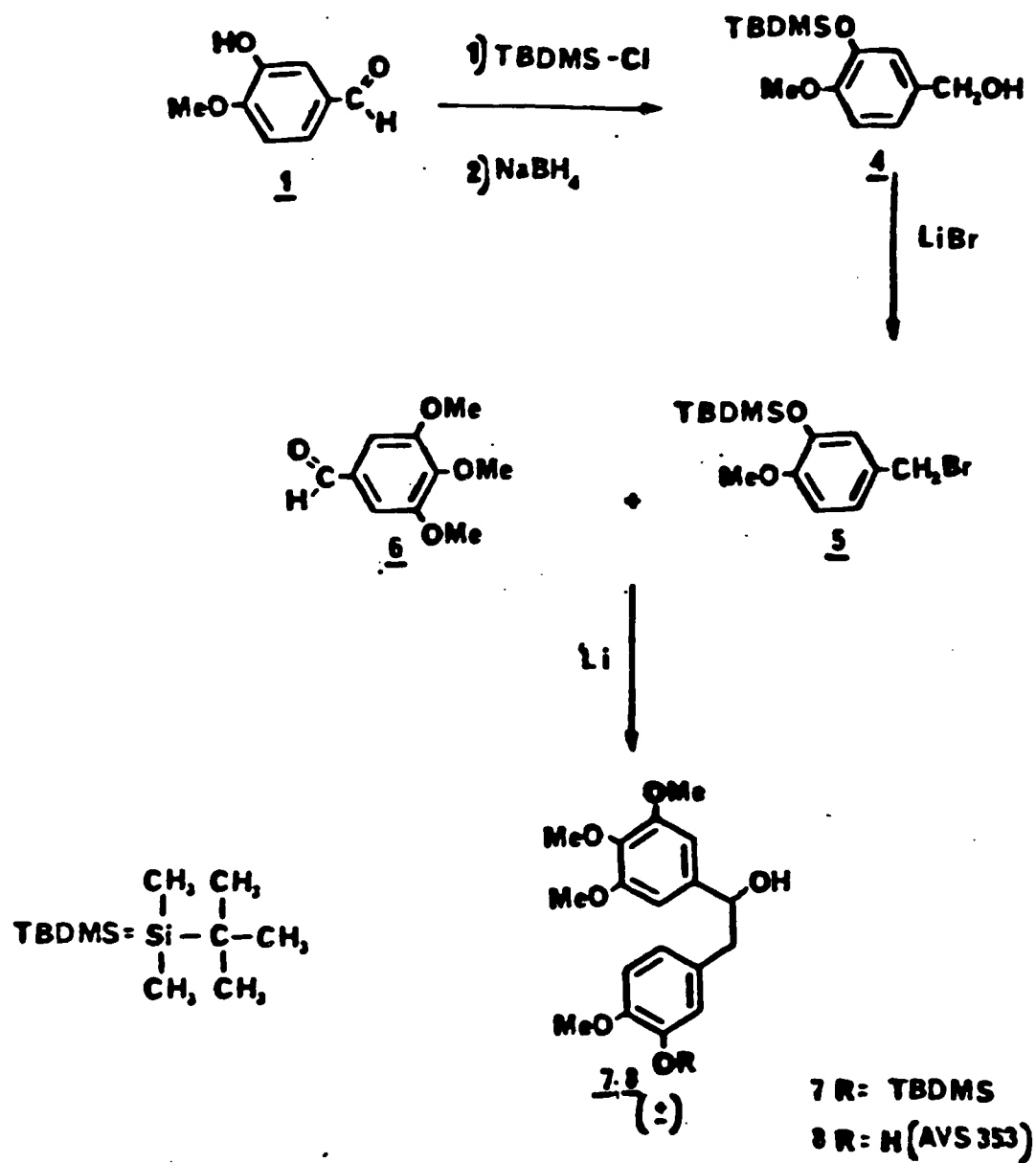
9-[5'-O-(t-Butyldimethylsilyl)- β -D-arabinofuranosyl]-adenine (2): To a stirred suspension of dry 9- β -D-arabinofuranosyl adenine (26.7 g, 0.1 mol) in dimethyl formamide (500 mL) is added imidazole (16.3 g, 0.24 mol), followed by the addition of t-butyldimethylsilyl chloride (18.1 g, 0.12 mol). The reaction mixture is stirred for 20 hours maintaining anhydrous conditions, then the solvent is removed by distillation under reduced pressure. The residue is treated with ethyl acetate (500 mL); the organic layer is washed with water, dried and evaporated. The obtained syrup is dissolved in chloroform (240 mL), the solution is warmed and hexane (200 mL) is added to give a turbid solution. After cooling to room temperature the obtained crystals are collected by filtration to give 33 g of 2. m.p. 157-158°.

9-(2',3'-Di-O-acetyl- β -D-arabinofuranosyl)adenine (4): Acetic anhydride (12.7 mL, 0.125 mol) is added dropwise to a stirred suspension of 9-[5'-O-(t-butyldimethylsilyl)- β -D-arabinofuranosyl]adenine (22.8 g, 0.06 mol) in pyridine (300 mL) at 0°. The mixture is stirred an additional 48 hours, when TLC indicates completion. Excess acetic anhydride is deactivated by adding a few milliliters of water, the solvent is evaporated under reduced pressure and the obtained syrup is dissolved in dichloromethane (500 mL). The organic layer is washed with water (2 x 100 mL), saturated sodium bicarbonate solution (100 mL) and water (100 mL). After drying over sodium sulfate the solvent is evaporated to leave 25.5 g of 3 as a solid foam. The solid is dissolved in a mixture of acetic acid (250 mL), tetrahydrofuran (100 mL) and water (100 mL) at 40° and stirred for 48 hours to remove the t-butyldimethylsilyl group. The solvent is evaporated and the resulting crude product is purified on a silica gel column, using dichloromethane-methanol 20:1 as the eluant. After evaporating the fractions containing diacetylarabino adenine the obtained solid is recrystallized from acetone to yield 10.5 g (43%) of pure product 4. m.p. 171-173° (literature m.p. 138-139° for product that contained 0.2 mol water of crystallization).

Starting with 50 g arabinoadenosine and following the same procedure, 24.0 g of final product had been submitted.

F. Combretastatin. AVS 353

AVS 353 was synthesized according to the following scheme:



Experimental⁶⁾

3-[(t-Butyldimethylsilyl)oxyl]-4-methoxybenzaldehyde (3): Isovanillin (1) (99.8 g, 0.656 mol) is dissolved in dimethylformamide (900 mL). After cooling the solution in an ice bath, t-butyldimethylsilyl chloride (117 g, 0.776 mol) is added, the ice bath is removed, and the reaction mixture is stirred for one hour, when thin layer chromatography indicates completion (silica gel; hexane-acetone 4:1). Water (100 mL) is added while stirring is continued, and after 10 minutes sodium bicarbonate solution (30 g sodium bicarbonate in 700 mL water) and ether (1200 mL) are added. The layers are separated, the aqueous layer is washed with ether (2 x 500 mL), and the combined ether layers are washed with brine (600 mL) and water (500 mL). The ether phase is dried over anhydrous sodium sulfate and upon evaporation of the ether 174 g (99.7%) of product 3 is obtained. The product is homogeneous by thin layer chromatography; single spot at Rf 0.55, hexane-acetone 4:1.

3-[(t-Butyldimethylsilyl)oxyl]-4-methoxybenzyl alcohol (4): 3-[(t-Butyldimethylsilyl)oxy]-4-methoxybenzaldehyde (3) (172 g, 0.647 mol) is dissolved in ethanol (1700 mL) and the solution is cooled in an ice bath. Sodium borohydride (27.0 g, 0.71 mol) is added in one portion, the ice bath is removed, and after stirring for 20 minutes thin layer chromatography indicates completion of reduction. Water (200 mL) is added and the volume is reduced to about 800 mL by distillation under diminished pressure. After adding 2 N sodium hydroxide solution (240 mL) the resulting layers are separated and the organic layer is dissolved in ether (800 mL). The ether layer is washed with water (2 x 250 mL), dried over anhydrous sodium sulfate and evaporated to leave the benzyl alcohol derivative 4 as a viscous liquid. Yield 116.9 g (67.4%).

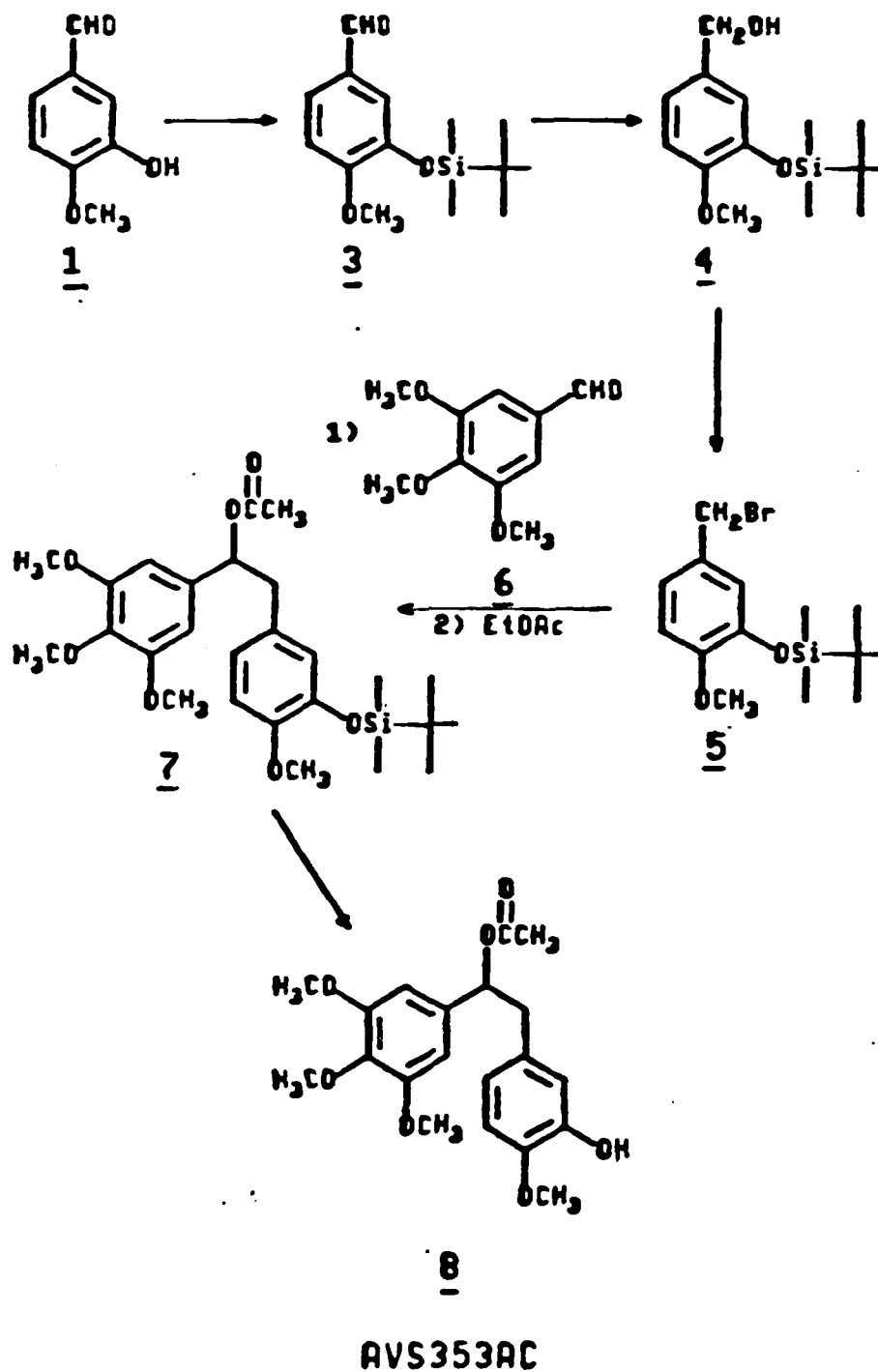
5-[(t-Butyldimethylsilyl)oxyl]-(+)-combretastatin (7): Under argon atmosphere chlorotrimethylsilane (10.0 mL, 79 mmol) is added to a vigorously stirred suspension of lithium bromide (6.0 g, 69 mmol) in acetonitrile (70 mL). Benzyl alcohol 4 (8.0 g, 30 mmol) in acetonitrile (30 mL) is added and the reaction mixture is stirred for one hour. Completion of the reaction is indicated by a single spot at Rf 0.8 in its thin layer chromatogram (silica gel; hexane/acetone 4:1). Ether (150 mL) is added and the solution is washed with water (2 x 30 mL), sodium bicarbonate 2% solution (100 mL), sodium hydroxide 5% (100 mL) and water (2 x 50 mL). The organic phase is dried over sodium sulfate and the solvent is evaporated under reduced pressure at room temperature to leave 3-[(t-butyldimethylsilyl)-4-methoxybenzylbromide (5) as an oil. Intermediate 5 is immediately dissolved in tetrahydrofuran (80 mL) together with 3,4,5-trimethoxybenzaldehyde (6) (2.2 g, 11 mmol). This solution is added to lithium shot 0.8 g, 115 mmol) in tetrahydrofuran (60 mL) over a 15 minute period.

After agitation with ultrasound in a sonicator the reaction reaches completion after 3 hours. The excess lithium metal is removed by filtration, and upon evaporation of the filtrate a viscous oil is obtained, consisting mainly of silylated combretastatin 7. Chromatographic purification on a silica gel column (200 g silica gel; first with 4 L hexane/ethyl acetate 4:1; then with 1 L hexane/ethyl acetate 3:1) leaves 2.2 g of 7 as a yellow, viscous oil. This product is used in the final step without further purification.

(+)-Combretastatin (8): Silyl ether 7 (6.8 g) is dissolved in dry tetrahydrofuran (120 mL), and during a 10 minute period tetrabutylammonium fluoride solution 1 molar in tetrahydrofuran (55 mL) is added. After an additional 10 minute reaction period the desilylation of 7 is complete, and the tetrahydrofuran is evaporated under reduced pressure. To the residue is added water (40 mL), the aqueous phase is extracted with ether (5 x 100 mL), the combined ether layers are dried and evaporated to leave 4.5 g of crude (+) Combretastatin (8). Combined batches of crude combretastatin (12.8 g) are loaded on a silica gel column (30 x 6 cm) and chromatographed using hexane/ethyl acetate 2:1 (3 L) and hexane/ethyl acetate 1:1 as the eluants. Upon evaporation of the phase containing pure 8, 10 g of pure Combretastatin is obtained as an amorphous solid; m.p. 105-106.5°; lit 103-105°.

G. Acetyl-combretastatin, AVS 353 AC

AVS 353 AC was synthesized according to the following scheme:



Experimental⁶⁾

3-[(t-Butyldimethylsilyl)oxy]-4-methoxybenzaldehyde (3): Isovanillin (1) (99.8 g, 0.656 mol) is dissolved in dimethylformamide (900 mL). After cooling the solution in an ice bath, t-butyldimethylsilyl chloride (117 g, 0.776 mol) is added, the ice bath is removed, and the reaction mixture is stirred for one hour, when thin layer chromatography indicates completion (silica gel; hexane-acetone 4:1). Water (100 mL) is added while stirring is continued, and after 10 minutes sodium bicarbonate solution (30 g sodium bicarbonate in 700 mL water) and ether (1200 mL) are added. The layers are separated, the aqueous layer is washed with ether (2 x 500 mL), and the combined ether layers are washed with brine (600 mL) and water (500 mL). The ether phase is dried over anhydrous sodium sulfate and upon evaporation of the ether 174 g (99.7%) of product 3 is obtained. The product is homogeneous by thin layer chromatography; single spot at Rf 0.55, hexane-acetone 4:1.

3-[(t-Butyldimethylsilyl)oxy]-4-methoxybenzyl alcohol (4): 3-[(t-Butyldimethylsilyl)oxy]-4-methoxybenzaldehyde (3) (172 g, 0.647 mol) is dissolved in ethanol (1700 mL) and the solution is cooled in an ice bath. Sodium borohydride (27.0 g, 0.71 mol) is added in one portion, the ice bath is removed, and after stirring for 20 minutes thin layer chromatography indicates completion of reduction. Water (200 mL) is added and the volume is reduced to about 800 mL by distillation under diminished pressure. After adding 2 N sodium hydroxide solution (240 mL) the resulting layers are separated and the organic layer is dissolved in ether (800 mL). The ether layer is washed with water (2 x 250 mL), dried over anhydrous sodium sulfate and evaporated to leave the benzyl alcohol derivative 4 as a viscous liquid. Yield 116.9 g (67.4%).

1-Acetoxy-1-(3,4,5-trimethoxyphenyl)-2-[3'-(tert-butyldimethylsilyl)oxy-4'-methoxyphenyl]ethane (7): Under argon atmosphere chlorotrimethylsilane (8.8 mL, 69 mmol) is added to a vigorously stirred suspension of lithium bromide (4.6 g, 53 mmol) in acetonitrile (60 mL). Benzyl alcohol 4 (7.0 g, 26 mmol) in acetonitrile (30 mL) is added and the reaction mixture is stirred for one hour. Completion of the reaction is indicated by a single spot at Rf 0.8 in its thin layer chromatogram (silica gel; hexane/acetone 4:1). Ether (150 mL) is added and the solution is washed with water (2 x 50 mL), sodium bicarbonate 2% solution (100 mL), sodium hydroxide 5% (100 mL) and water (2 x 50 mL). The organic phase is dried over sodium sulfate and the solvent is evaporated under reduced pressure at room temperature to leave 3[(t-butyldimethylsilyl)-4-methoxy-benzylbromide (5) as an oil. Intermediate 5 is immediately dissolved in tetrahydrofuran (80 mL) together with 3,4,5-trimethoxybenzaldehyde (6) (2.2 g, 11 mmol). This solution is added to lithium shot (0.7 g, 0.1 mmol) in tetrahydrofuran (60 mL) over a 15 minute period. After agitation with ultrasound in a sonicator the reaction reaches completion after 3½ hours. The excess lithium is removed by filtration, silica gel (40 g) and ethyl acetate (50 mL) are added, and the solvent is evaporated to leave the coated silica gel. Exposure of the formed coupling product to excess ethyl acetate, in the presence of activated silica gel, causes transesterification with the hydroxyl group at C-1 to

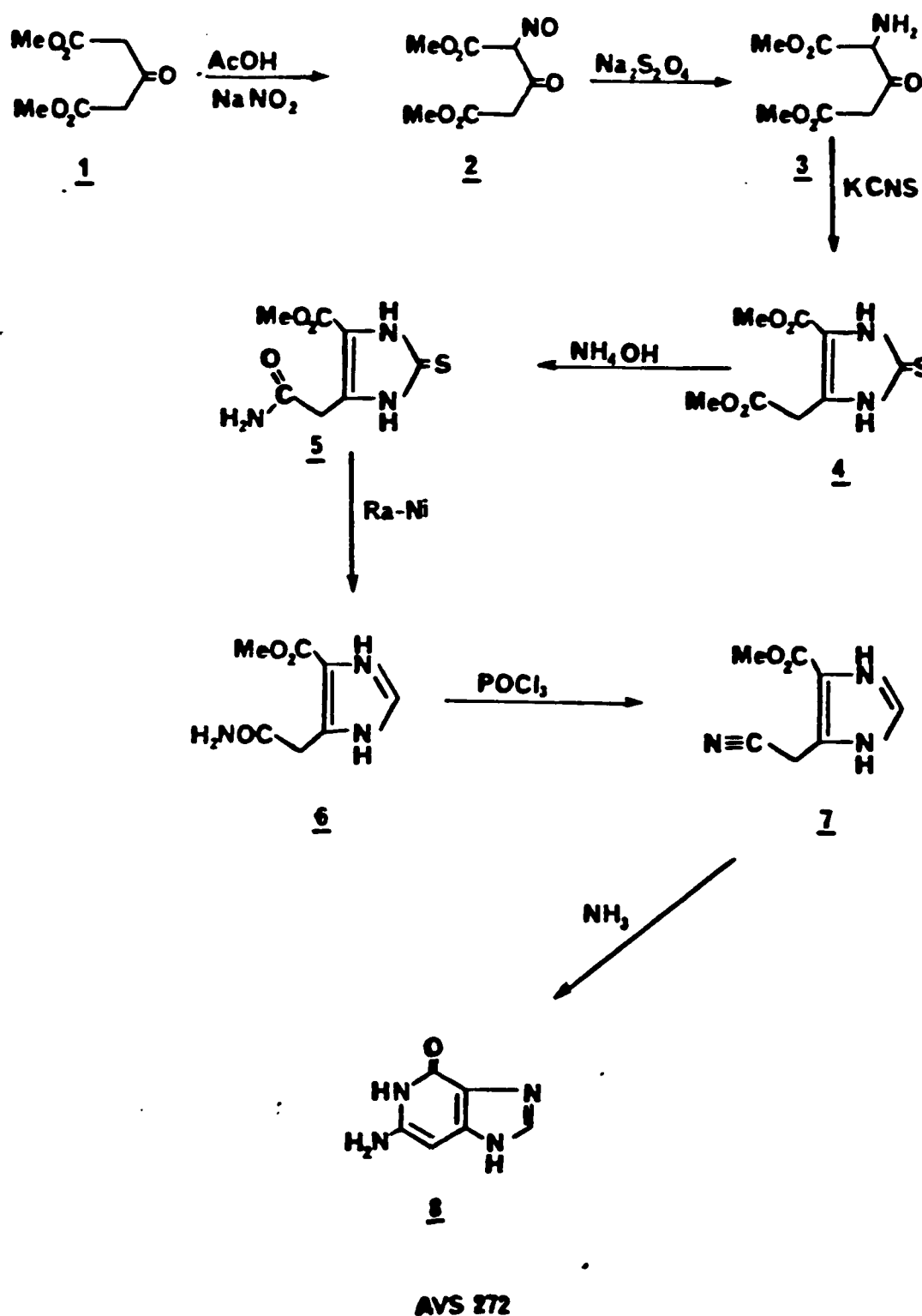
AVS 353 AC

produce 7 as the acetate. Column chromatographic elution with hexane:ethyl acetate 4:1 (5 L) produces 2.6 g of 7, obtained as a yellow solid after evaporation of the solvent under reduced pressure.

(+)-Combretastatin - acetate (8): Silyl ether 7 (1.7 g) is dissolved in dry tetrahydrofuran (30 mL), and during a 10 minute period tetrabutylammonium fluoride solution 1 molar in tetrahydrofuran (16 mL) is added. After an additional 10 minute reaction period the desilylation of 7 is complete, and the tetrahydrofuran is evaporated under reduced pressure. To the residue is added water (40 mL), the aqueous phase is extracted with ether (3 x 100 mL), the combined ether layers are dried and evaporated to leave crude (+) Combretastatin acetate (8). The solid is recrystallized from ethanol (30 mL) to yield 1.30 g (89.2%) of Combretastatin acetate.

H. 3-Deazaguanine, AVS 272

AVS 272 was synthesized according to the following scheme:



Experimental⁷⁾

4-Acetic acid-2-imidazolethione-5-carboxylic acid dimethyl ester (4): To methyl acetone dicarboxylate (128 g, 0.73 mol) in glacial acetic acid (160 mL) is added a sodium nitrite solution (43.2 g, 0.62 mol, in 64 mL water) while stirring. The temperature is kept between 25-35°, stirring is maintained for 30 minutes, and upon addition of water (1400 mL) stirring is continued for one hour. Sodium dithionite (384 g, 2.4 mol) is added portionwise, and the mixture is stirred until all the solid is dissolved. The pH is adjusted to 4 with dilute sulfuric acid, and potassium thiocyanide (184 g, 1.9 mol) is added in small portions. Upon completion of thiocyanide addition the solution is allowed to stand for 20 minutes, then heated until it reaches 70°C. After standing overnight at 0° the precipitated yellow crystals are collected by filtration and recrystallized from water/methanol. Yield 43 g; m.p. 220-222°; lit 220-222°

4-Acetamide-2-imidazolethione-5-carboxylic acid methyl ester (5): Imidazole derivative 4 (200 g, 0.87 mol) is added portionwise to ammonium hydroxide (2210 mL, from 1420 mL aqueous 38% ammonium hydroxide and 790 mL water), then heated to 95-100°. After all the product has dissolved the dark solution is kept at reflux five more minutes, then cooled in an ice bath and adjusted to pH 3 with dilute sulfuric acid. Upon standing overnight at 0° the precipitated crystals are collected by filtration and dried. Yield 114 g, m.p. 221°; lit 233° dec.

4-Imidazole-acetamide-5-carboxylic acid methyl ester (6): Moist Raney nickel (150 g) is suspended with stirring in ethanol (1 L), imidazole thione intermediate 5 (40 g, 0.18 mol) is added portionwise, and the reaction mixture is kept at reflux for two hours. After collecting the Raney nickel by filtration the filtrate is evaporated to dryness under reduced pressure and the residue is recrystallized from hot water (150 mL) to yield 13.9 g (40%) of desulfurized compound 6. m.p. 234°; lit 242-244°.

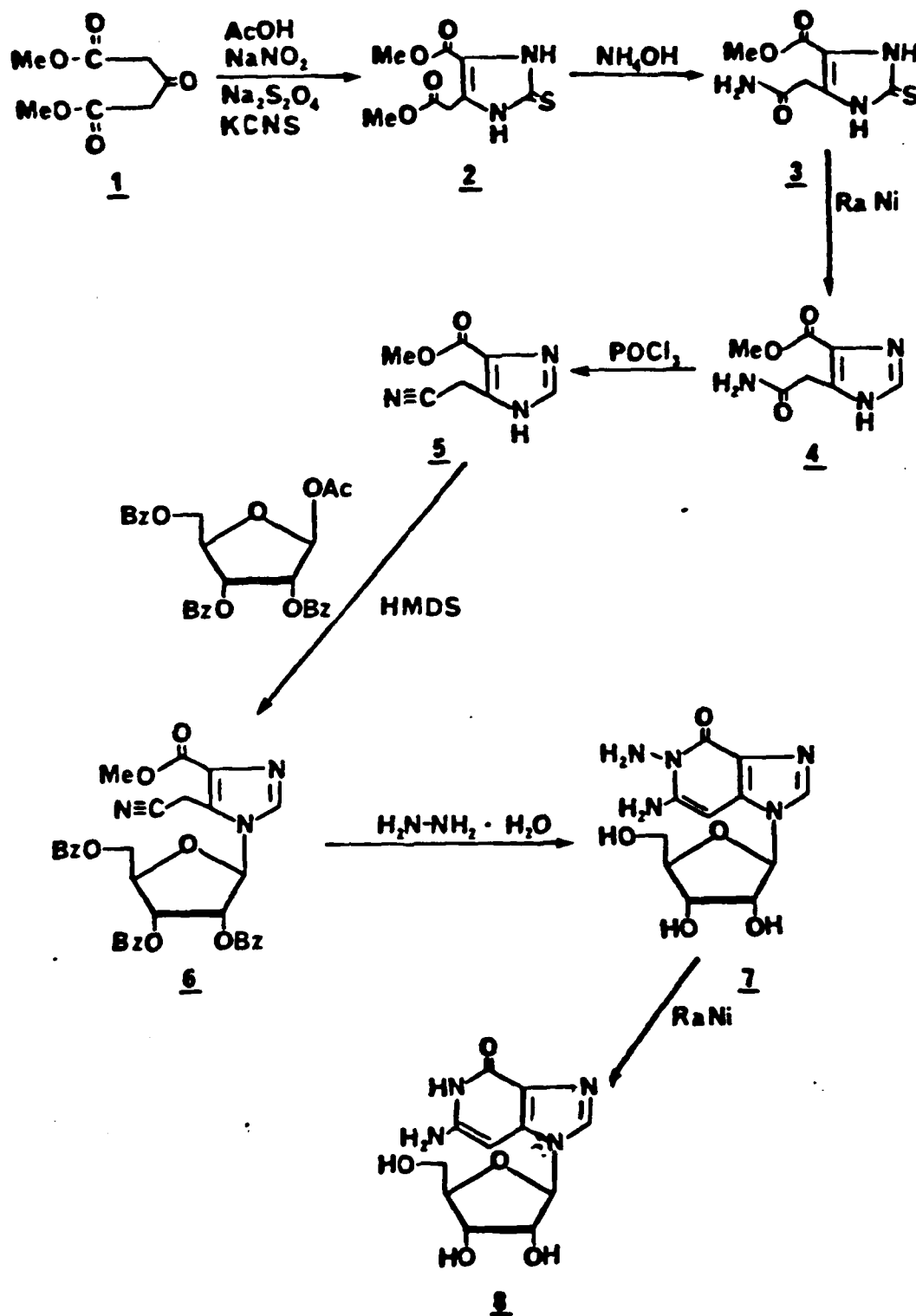
Methyl-5(4)-cyanomethylimidazole-4(5)-carboxylate (7): 4-Acetamido-imidazole-5-methyl carboxylate 6 (14.6 g, 80 mmol) is suspended in a mixture of triethylamine (120 mL) and dichloromethane (200 mL). Upon cooling below 10° phosphorus oxychloride (26.4 mL, 0.28 mol) is added to the stirred reaction mixture over a thirty minute period. After removal of the ice bath stirring is continued at room temperature for four hours, then the reaction mixture is chilled in an acetone-dry ice bath and water (20 mL) is cautiously added. After stirring an additional hour at room temperature the dichloromethane is removed under reduced pressure, the aqueous phase is adjusted to pH 7 by cautious addition of small drops of concentrated ammonium hydroxide solution, and thoroughly extracted with ethyl acetate (5 x 200 mL). The organic phase is washed with water, dried with anhydrous sodium sulfate and evaporated under reduced pressure to yield 5.4 g (40%) of cyanomethylimidazole-carboxylate 7. m.p. 168-170°; lit 170-171°.

AVS 272

3-Deazaguanine (8): Methyl-5-cyanomethylimidazole-4-carboxylate 7 (16.5 g, 0.1 mol) is placed in a steel bomb containing liquid ammonia (150 g). The vessel is sealed and kept at 100° for eight days. Upon cooling excess ammonia is allowed to slowly evaporate, then the solid residue is treated with decolorizing charcoal in hot water and recrystallized. Yield: 9.0 g (66%); m.p. > 350°C.

I. 3-Deazaguanosine, AVS 215

AVS 215 was synthesized according to the following scheme:



AVS 215

Experimental^a

4-Acetic acid-2-imidazolethione-5-carboxylic acid dimethyl ester (2): To methyl acetone dicarboxylate (128 g, 0.73 mol) in glacial acetic acid (160 mL) is added a sodium nitrite solution (43.2 g, 0.62 mol, in 64 mL water) while stirring. The temperature is kept between 25-35°, stirring is maintained for 30 minutes, and upon addition of water (1400 mL) stirring is continued for one hour. Sodium dithionite (384 g, 2.4 mol) is added portionwise, and the mixture is stirred until all the solid is dissolved. The pH is adjusted to 4 with dilute sulfuric acid, and potassium thiocyanide (184 g, 1.9 mol) is added in small portions. Upon completion of thiocyanide addition the solution is allowed to stand for 20 minutes, then heated until it reaches 70°C. After standing overnight at 0° the precipitated yellow crystals are collected by filtration and recrystallized from water/methanol. Yield 43 g; m.p. 220-222°; lit 220-222°

4-Acetamide-2-imidazolethione-5-carboxylic acid methyl ester (3): Imidazole derivative 4 (200 g, 0.87 mol) is added portionwise to ammonium hydroxide (2210 mL, from 1420 mL aqueous 38% ammonium hydroxide and 790 mL water), then heated to 95-100°. After all the product has dissolved the dark solution is kept at reflux five more minutes, then cooled in an ice bath and adjusted to pH 3 with dilute sulfuric acid. Upon standing overnight at 0° the precipitated crystals are collected by filtration and dried. Yield 114 g, m.p. 221°; lit 233° dec.

4-Imidazole-acetamide-5-carboxylic acid methyl ester (4): Moist Raney nickel (150 g) is suspended with stirring in ethanol (1 L), imidazole thione intermediate 3 (40 g, 0.18 mol) is added portionwise, and the reaction mixture is kept at reflux for two hours. After collecting the Raney nickel by filtration the filtrate is evaporated to dryness under reduced pressure and the residue is recrystallized from hot water (150 mL) to yield 13.9 g (40%) of desulfurized compound 4. m.p. 234°; lit 242-244°.

Methyl-5(4)-cyanomethylimidazole-4(5)-carboxylate (5): 4-Acetamido-imidazole-5-methyl carboxylate (4) (14.6 g, 80 mmol) is suspended in a mixture of triethylamine (120 mL) and dichloromethane (200 mL). Upon cooling below 10° phosphorus oxychloride (26.4 mL, 0.28 mol) is added to the stirred reaction mixture over a thirty minute period. After removal of the ice bath stirring is continued at room temperature for four hours, then the reaction mixture is chilled in an acetone-dry ice bath and water (20 mL) is cautiously added. After stirring an additional hour at room temperature the dichloromethane is removed under reduced pressure, the aqueous phase is adjusted to pH 7 by cautious addition of small drops of concentrated ammonium hydroxide solution, and thoroughly extracted with ethyl acetate (5 x 200 mL). The organic phase is washed with water, dried with anhydrous sodium sulfate and evaporated under reduced pressure to yield 5.4 g (40%) of cyanomethylimidazole-carboxylate 5; m.p. 168-170°; lit 170-171°.

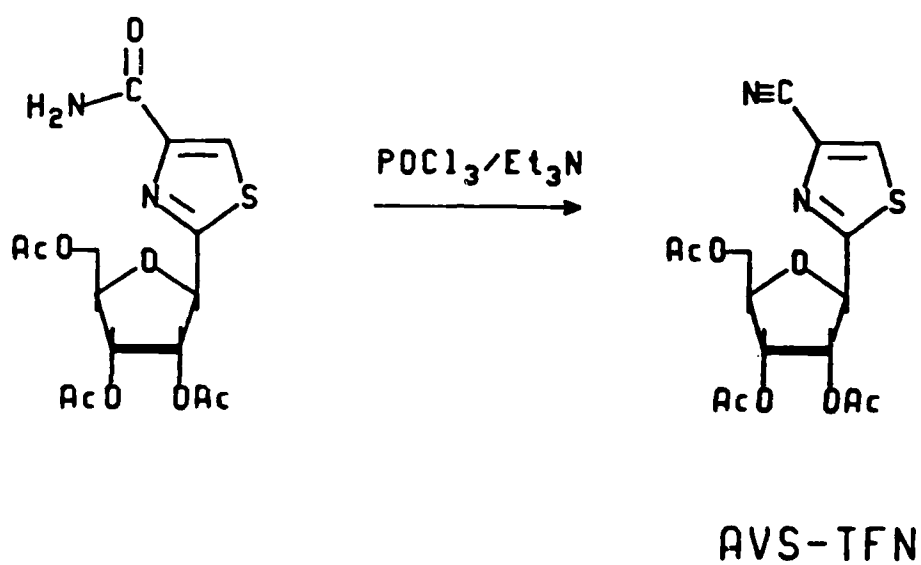
Methyl-5-cyanomethyl-1-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)-imidazole-4-carboxylate (6): Cyanomethylimidazole carboxylate 5 (12.5 g, 75 mmol) is combined with hexamethyldisilazane (150 mL) under anhydrous conditions and kept at reflux for 12 hours in the presence of a catalytic amount of ammonium sulfate (500 mg). After removing excess hexamethyldisilazane by distillation under reduced pressure the silyl derivative of 5 is obtained as an oil, which is dissolved in 1,2-dichloromethane (400 mL). Acetyl-2,3,5-tri-O-benzoyl-ribofuranose (38 g, 75 mmol) is added, followed by stannic chloride (12.7 mL, 108 mmol). The reaction mixture is agitated for 24 hours, then it is poured into a 5% sodium bicarbonate solution (1.5 L). After filtering through a Celite pad, the organic layer is separated and the aqueous phase is extracted with dichloromethane (2 x 800 mL). The combined organic layers are dried over anhydrous sodium sulfate, and upon removal of the solvent under reduced pressure nucleoside 6 is obtained as a cream-colored solid foam. The compound is purified by passing it through a silica gel column, with chloroform/acetone 4:1 as the eluant. After evaporation of the solvent 30.0 g (66%) of pure 6 is obtained. m.p. 95-100°.

1-Amino-3-deazaguanosine (7): Hydrazine monohydrate (23 mL, 0.66 mol) is added to a solution of ribofuranosyl-imidazole-carboxylate 6 (28 g, 46 mmol) dissolved in absolute ethanol (250 mL). The reaction mixture is heated to reflux when after about two hours a solid starts to separate. Heating is continued an additional 20 hours, and upon cooling the precipitate is collected by filtration, and washed with ethanol. Yield: 8.4 g (61%), m.p. 254-255°; lit. 254-255°.

3-Deazaguanosine (8): 1-Amino-3-deazaguanosine (27 g, 91 mmol) is dissolved in deionized water (2 L) and heated to reflux. Raney Nickel (240 g aqueous slurry) is added in small portions over a 4 hour period. Heating is continued for 30 minutes, the nickel is collected on a Celite filter pad, and the filtrate is concentrated to give 3-deazaguanosine as a white precipitate. Yield: 17.5 g (68%); m.p. 256-258°; lit. 255-257°.

J. Thiazofurin-nitrile, AVS TFN

AVS TFN was synthesized according to the following scheme:



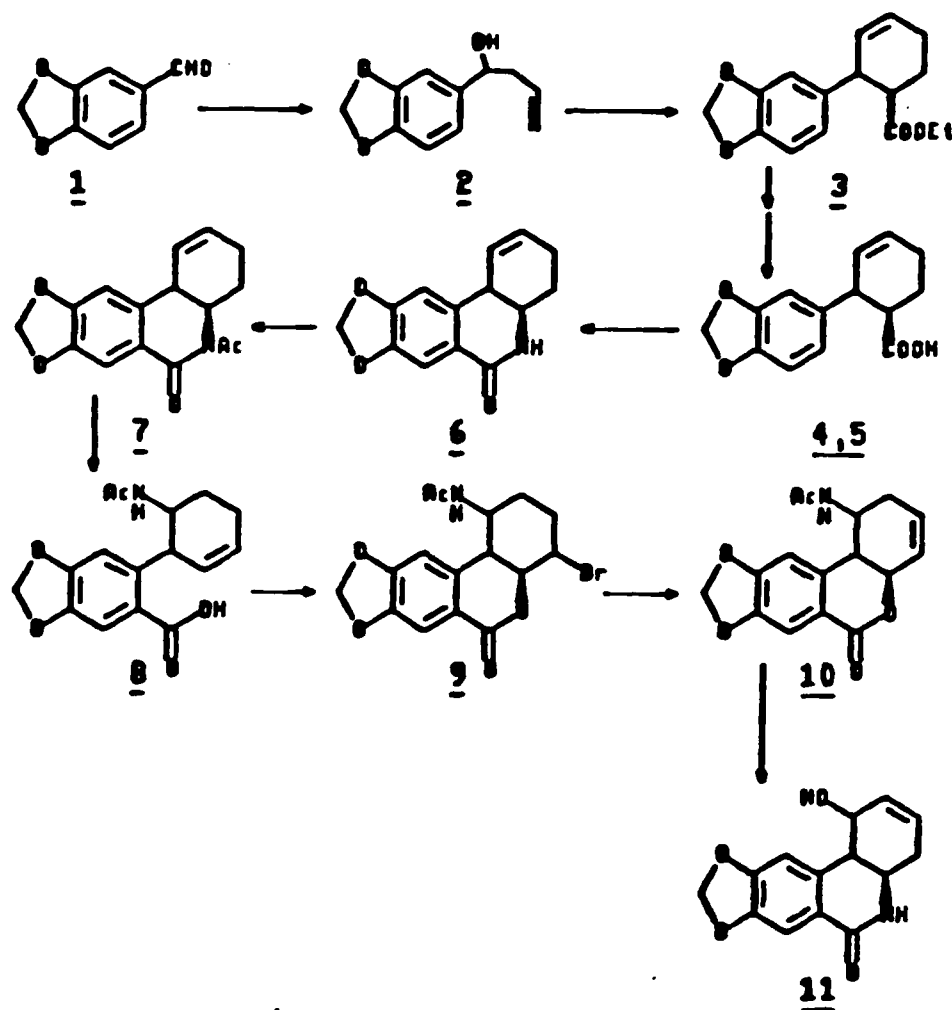
AVS TFN

2-(2,3,5-Tri-O-acetyl- β -D-ribofuranosyl)thiazole-4-carboxamide:

Experimental: A solution of thiazofurin (19.3 g, 50 mmol) in methylene chloride (400 mL) is cooled to 0°C, triethylamine (102.7 mL) is added, followed by addition of phosphorus oxychloride (13 mL, 137 mmol). During a two hour period the solution is allowed to warm to room temperature while stirring, then the solvent is removed under reduced pressure. The oily residue is suspended in water (500 mL) and extracted with methylene chloride (2 x 250 mL). The combined methylene chloride extracts are washed with water and dried over sodium sulfate. After evaporation of the solvent the obtained syrup is passed through a silica gel column, with methylene chloride containing 5% acetone as the mobile phase. The fractions containing the desired product are combined, the solvent is evaporated and the product is obtained as a viscous syrup, as reported in the literature. Yield 16.5 g (90.5%).

K. 4aH-r.1H-trans.1-Hydroxy-8,9-methylenedioxy-1,4,4a,10b-tetrahydro-6(5H)-phenanthridone. Intermediate 11

Intermediate 11 was synthesized according to the following scheme:



Intermediate 11

Experimental^o

Ethyl-trans-2-(3',4'-methylenedioxyphenyl)-3-cyclohexene-1-carboxylic acid (3): Piperonal (500 g, 3.33 mol) is dissolved in tetrahydrofuran (3 L), and the solution is cooled below 5° in an ice bath. Allylmagnesium chloride solution (2000 mL, 4.0 mol) is added over a 2½ hour period while maintaining the temperature below 5°. After completion of addition, the reaction mixture is stirred an additional 30 minutes at low temperature. Then it is allowed to warm up to room temperature and kept there for three hours.

After cooling again below 5° a saturated ammonium chloride solution (1 L) is added during a 40 minute interval. The organic layer is separated and washed with brine (4 L) several times, and the aqueous layer is washed with chloroform (1 L). The combined organic layers are dried over sodium sulfate, filtered, and the solvent is removed under reduced pressure. Upon eliminating any remaining solvent under high vacuum, the resulting oil is applied in the subsequent reaction without further purification.

The obtained allyl carbinol derivative 2 (309 g) ethyl acrylate (195 g) and p-toluene sulfonic acid (54 g) are kept in a sealed bomb at 175-185° for six hours. Upon cooling the reaction mixture is submitted to distillation under reduced pressure to eliminate excess ethyl acrylate. The residue is dissolved in ether (3 L), the organic layer is washed with water (1 L), 5% sodium bicarbonate solution (1 L), and water (1 L). After drying with sodium sulfate overnight the solvent is taken off under reduced pressure and the residual oil is fractionally distilled where the desired product 3 boils at 145-155°/0.25 mm Hg, yield 263 g (90%). (Overall yield 29%)

trans-2-(3',4'-Methylenedioxyphenyl)-3-cyclohexene-1-carboxylic acid (4): A solution of sodium metal (10 g, 0.435 mol) in ethanol (300 mL) is combined with a solution of phenyl ester 3 in ethanol (300 mL), and the resulting mixture is kept at reflux for 2 hours. Water (60 mL) is added to the reaction mixture and reflux is continued for 4 more hours. The ethanol is evaporated under reduced pressure, the residue is dissolved in water (1 L) and washed with ether (3 x 300 mL). Upon cooling the aqueous layer, the pH is adjusted to pH 2 with conc. hydrochloric acid (prox. 25 mL), stirred for 30 minutes, and the precipitate is collected by filtration. The product is washed with water and air-dried to yield 81.1 g (90.3%) of acid 4; m.p. 101-102°, lit. 102-103°.

4aH-r.10bH-trans-8,9-Methylenedioxy-3,4,4a,10b-tetrahydro-6(5H)-phenanthridone (6): Carboxylic acid 4 (200 g, 0.81 mol) is dissolved in acetone (1 L), water (200 mL) and triethylamine (82 g, 0.81 mol) is added, and the mixture is cooled in an ice bath. A solution of ethyl chloroformate (87.4 g, 0.81 mol) in acetone (200 mL) is added slowly while stirring. Two hours after completion a solution of sodium azide (80 g, 1.23 mol) in water (200 mL) is added slowly during a one hour interval, followed by two hours of stirring at the lowered temperature. A mixture of toluene (1 L) and water (1.5 L) is added and stirring is continued for an additional 1.5 hours. The organic layer is separated, the aqueous layer washed with toluene (300 mL), and the combined organic layers are washed with water (2 x 1 L) and dried over sodium sulfate.

Intermediate 11

After filtering, the obtained solution is concentrated to 1700 mL under diminished pressure at 45°, followed by maintaining the concentrated solution at reflux for 4 hours. When TLC shows the disappearance of starting material the solution is cooled, and the solvent is evaporated under reduced pressure to leave 5 as a light tan colored oil, which is used in the next step without further purification.

The oily product 5 is cooled in an ice-water bath and carefully treated with boron trifluoride etherate (400 mL, 3.0 mol). The reaction mixture is allowed to stand at room temperature overnight. The crystalline solid that separates is filtered, washed with ether (3 x 200 mL) and dried in air; yield 155.0 g. The filtrate is evaporated to dryness under high vacuum, the residue is dissolved in ether (400 mL) and washed with ice-cold water (2 x 500 mL). The organic layer is dried over anhydrous sodium sulfate and evaporated to dryness. The obtained residue is again treated with boron trifluoride etherate (25 mL) and worked up as previously described to yield 4.4 g of additional product, which is identical to the main crop and hence they are combined. Total yield: 159.4 g (80.9%); m.p. 220-221° lit 220°.

4aH-r-10bH-trans-5-Acetyl-8,9-methylenedioxy-3,4,4a,10b-H-tetrahydro-6(5H)phenanthridone (7): A mixture of tetrahydrophenanthridone 6 (50.0 g, 0.206 mol), acetic anhydride (500 mL) and DMAP (1.0 g) is refluxed for 4 hours. Upon cooling a solid precipitates from solution. The precipitate is collected by filtration and sucked to dryness. The filtrate is evaporated under reduced pressure (40°), the resulting solid is triturated with ethanol, filtered, and the filter cake is combined with the one obtained previously. After an additional trituration of the combined product with ethanol, the solid is filtered off, washed with ice-cold ethanol and air dried. Yield: 40.0 g (62%); m.p. 155-157°, lit. 157-158°.

4H-r,3H-trans,3-(3',4'-Methylenedioxy-6'-carboxyphenyl)-4-acetamino-1-cyclohexene (8): N-acetylphenanthridone 7 (60.0 g, 0.2 mol) is suspended in methanol (1 L), then a solution of potassium hydroxide (22.4 g, 0.4 mol) in water (400 mL) is added, and the flask is immersed in a water bath preheated to 80°. The reaction mixture reaches a temperature of 70°C, and the reaction is kept at that temperature for 15 minutes. When cooled to room temperature a white solid starts to separate. The volume of the reaction mixture is reduced to 400 mL and the resulting precipitate is collected by filtration, washed with water (2 x 25 mL) and dried in air. It can be shown that the obtained precipitate is starting material 7.

The filtrate is cooled in an ice bath and acidified with con. hydrochloric acid to form a white precipitate that is filtered off, washed with water (2 x 25 mL) and dried in air. This product agrees in its spectral and analytical data with the structural assignment for 8, hence it is taken in the next step without further purification. Yield: 40.0 g (62%); m.p. 200-201°, lit. 198-201°. Recovered starting material: 20.5 g (37.0%).

Intermediate 11

4aH-r,1H-trans,10bH-cis,4H-trans,1-Acetylamino-4-bromo-8,9-methylenedioxy-1,2,3,4,4a,10b-hexahydrobenzo[b,d]pyrone-6 (2): To a well-stirred suspension of acetylamino carboxylic acid 8 (145.3 g, 0.48 mol) in tetrahydrofuran (2 L) powdered N-bromosuccinimide (89.0 g, 0.497 mol) is added in a single portion. Most of the solid dissolves, and a crystalline material begins to separate. After stirring for 1 hour the reaction mixture is cooled in an ice bath, then the precipitate is collected by filtration, washed with cold tetrahydrofurane, and dried. Yield: 175.5 g (93.3%); m.p. 265-267°, lit. 270°.

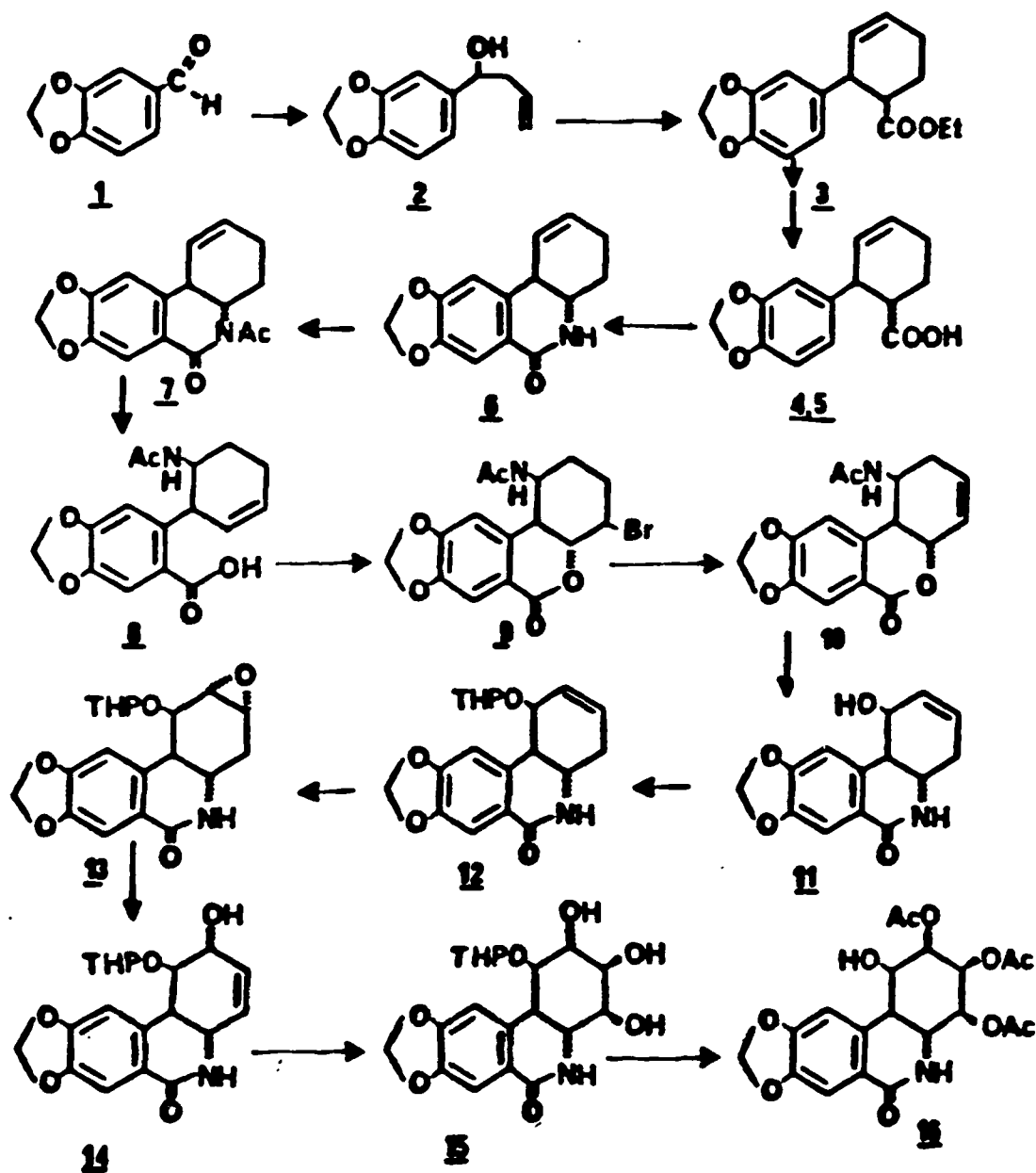
4aH-r,1H-trans,10bH-cis,1-Acetylamino-8,9-methylenedioxy-1,2,4a,10b-tetrahydrodibenzo[b,d]pyrone-6 (10): A mixture of acetylaminobromolactone 2 (456.1 g, 1.163 mol) and DBU (182 g, 1.2 mol) in pyridine (5.0 L) is kept at reflux for 8½ hours under anhydrous conditions. After cooling overnight a crystalline material precipitates. The solid is filtered off and slurried with water for 10 minutes, filtered, and air-dried. The filtrate is evaporated to dryness at oil vacuum pressure, and the solid residue is slurried in water for 10 minutes, filtered, air-dried, and combined with the previous precipitate. Yield: 339.0 g (93.7%); m.p. 270-272°, lit. 263-267°.

4aH-r,1H-trans,1-Hydroxy-8,9-methylenedioxy-1,4,4a,10b-tetrahydro-6(5H)-phenanthridone (11): A mixture of acetylaminolactone 10 (60.0 g, 0.193 mol) and ethanol (150 mL) is treated with a solution of NaOH (30 g) in water (150 mL), then the reaction is heated to 90-95°C, and kept at that temperature for 8 hours. A small amount of water is added periodically to dissolve a solid that separates. The reaction mixture is allowed to cool to room temperature overnight, the precipitated solid is collected by filtration, washed with water and dried in air. The filtrate is acidified with concentrated hydrochloric acid, the resulting precipitate is filtered, washed with water (3 x 100 mL) and dried in air. Both precipitated solids are found to be identical therefore they are combined, however, NMR-analysis indicates the presence of substantial amounts of starting material. The product is redissolved in 20% sodium hydroxide (300 mL), kept at 120° for five hours, cooled, and reprecipitated. Repeating the procedure five times the obtained product does not show any more contamination. Yield: 94.0 g; m.p. 260-280° (dec.), lit. 265-280° (dec.).

A 200 mg purified sample was submitted for testing.

L. 4aH-r,1H-trans,2H-cis,3H-trans,4H-trans,1-Hydroxy,2,3,4-triacetoxy-8,9-methylenedioxy-1,2,3,4,4a,10b-hexahydro-6(5H)phenanthridone, AVS 360 OH

AVS 360 OH was synthesized according to the following scheme:



Experimental^{10,11}

Ethyl-trans-2-(3',4'-methylenedioxyphenyl)-3-cyclohexene-1-carboxylic acid (3): Piperonal (500 g, 3.33 mol) is dissolved in tetrahydrofuran (3 L), and the solution is cooled below 5° in an ice bath. Allylmagnesium chloride solution (2000 mL, 4.0 mol) was added over a 2½ hour period while maintaining the temperature below 5°. After completion of addition, the reaction mixture is stirred an additional 30 minutes at low temperature. Then it is allowed to warm up to room temperature and kept there for three hours.

After cooling again below 5° a saturated ammonium chloride solution (1 L) is added during a 40 minute interval. The organic layer is separated and washed with brine (4 L) several times, and the aqueous layer is washed with chloroform (1 L). The combined organic layers are dried over sodium sulfate, filtered, and the solvent is removed under reduced pressure. Upon eliminating any remaining solvent under high vacuum, the resulting oil is applied in the subsequent reaction without further purification.

The obtained allyl carbinol derivative 2 (309 g) ethyl acrylate (195 g) and p-toluene sulfonic acid (54 g) are kept in a sealed bomb at 175-185° for six hours. Upon cooling the reaction mixture is submitted to distillation under reduced pressure to eliminate excess ethyl acrylate. The residue is dissolved in ether (3 L), the organic layer is washed with water (1 L), 5% sodium bicarbonate solution (1 L), and water (1 L). After drying with sodium sulfate overnight the solvent is taken off under reduced pressure and the residual oil is fractionally distilled where the desired product 3 boils at 145-155°/0.25 mm Hg, yield 263 g (90%). (Overall yield 29%).

trans-2-(3',4'-Methylenedioxyphenyl)-3-cyclohexene-1-carboxylic acid (4): A solution of sodium metal (10 g, 0.435 mol) in ethanol (300 mL) is combined with a solution of phenyl ester 3 in ethanol (300 mL), and the resulting mixture is kept at reflux for 2 hours. Water (60 mL) is added to the reaction mixture and reflux is continued for 4 more hours. The ethanol is evaporated under reduced pressure, the residue is dissolved in water (1 L) and washed with ether (3 x 300 mL). Upon cooling the aqueous layer, the pH is adjusted to pH 2 with conc. hydrochloric acid (prox. 25 mL), stirred for 30 minutes, and the precipitate is collected by filtration. The product is washed with water and air-dried to yield 81.1 g (90.3%) of acid 4; m.p. 101-102°, lit. 102-103°.

4aH-r.10bH-trans-8,9-Methylenedioxy-3,4,4a,10b-tetrahydro-6(5H)-phenanthridone (6): Carboxylic acid 4 (200 g, 0.81 mol) is dissolved in acetone (1 L), water (200 mL) and triethylamine (82 g, 0.81 mol) is added, and the mixture is cooled in an ice bath. A solution of ethyl chloroformate (87.4 g, 0.81 mol) in acetone (200 mL) is added slowly while stirring. Two hours after completion a solution of sodium azide (80 g, 1.23 mol) in water (200 mL) is added slowly during a one hour interval, followed by two hours of stirring at the lowered temperature. A mixture of toluene (1 L) and water (1.5 L) is added and stirring is continued for an additional 1.5 hours. The organic layer is separated, the aqueous layer washed with toluene (300 mL), and the combined organic layers are washed with water (2 x 1 L) and dried over sodium sulfate.

After filtering, the obtained solution is concentrated to 1700 mL under diminished pressure at 45°, followed by maintaining the concentrated solution at reflux for 4 hours. When TLC showed the disappearance of starting material the solution is cooled, and the solvent is evaporated under reduced pressure to leave **5** as a light tan colored oil, which is used in the next step without further purification.

The oily product **5** is cooled in an ice-water bath and carefully treated with boron trifluoride etherate (400 mL, 3.0 mol). The reaction mixture is allowed to stand at room temperature overnight. The crystalline solid that separates is filtered, washed with ether (3 x 200 mL) and dried in air; yield 155.0 g. The filtrate is evaporated to dryness under high vacuum, the residue is dissolved in ether (400 mL) and washed with ice-cold water (2 x 500 mL). The organic layer is dried over anhydrous sodium sulfate and evaporated to dryness. The obtained residue is again treated with boron trifluoride etherate (25 mL) and worked up as previously described to yield 4.4 g of additional product, which is identical to the main crop and hence they are combined. Total yield: 159.4 g (80.9%); m.p. 220-221° lit 220°.

4aH-r-10bH-trans-5-Acetyl-8,9-methylenedioxy-3,4,4a,10b-H-tetrahydro-6(5H)phenanthridone (**7**): A mixture of tetrahydrophenanthridone **6** (50.0 g, 0.206 mol), acetic anhydride (500 mL) and DMAP (1.0 g) is refluxed for 4 hours. Upon cooling a solid precipitates from solution. The precipitate is collected by filtration and sucked to dryness. The filtrate is evaporated under reduced pressure (40°), the resulting solid is triturated with ethanol, filtered, and the filter cake is combined with the one obtained previously. After an additional trituration of the combined product with ethanol, the solid is filtered off, washed with ice-cold ethanol and air dried. Yield: 40.0 g (62%); m.p. 155-157°, lit. 157-158°.

4H-r.3H-trans.3-(3',4'-Methylenedioxy-6'-carboxyphenyl)-4-acetamino-1-cyclohexene (**8**): N-acetylphenanthridone **7** (60.0 g, 0.2 mol) is suspended in methanol (1 L), then a solution of potassium hydroxide (22.4 g, 0.4 mol) in water (400 mL) is added, and the flask is immersed in a water bath preheated to 80°. The reaction mixture reaches a temperature of 70°C, and the reaction is kept at that temperature for 15 minutes. When cooled to room temperature a white solid starts to separate. The volume of the reaction mixture is reduced to 400 mL and the resulting precipitate is collected by filtration, washed with water (2 x 25 mL) and dried in air. It can be shown that the obtained precipitate is starting material **7**.

The filtrate is cooled in an ice bath and acidified with con. hydrochloric acid to form a white precipitate that is filtered off, washed with water (2 x 25 mL) and dried in air. This product agrees in its spectral and analytical data with the structural assignment for **8**, hence it is taken in the next step without further purification. Yield: 40.0 g (62%); m.p. 200-201°, lit. 198-201°. Recovered starting material: 20.5 g (37.0%).

4aH-r.1H-trans.10bH-cis.4H-trans.1-Acetylamino-4-bromo-8.9-methylenedioxy-1.2.3.4.4a.10b-hexahydrobenzo[b,d]pyrone-6 (9): To a well-stirred suspension of acetylamino carboxylic acid 8 (145.3 g, 0.48 mol) in tetrahydrofuran (2 L) powdered N-bromosuccinimide (89.0 g, 0.497 mol) is added as a single portion. Most of the solid dissolves, and a crystalline material begins to separate. After stirring for 1 hour the reaction mixture is cooled in an ice bath, then the precipitate is collected by filtration, washed with cold tetrahydrofuran, and dried. Yield: 175.5 g (93.3%); m.p. 265-267°, lit. 270°.

4aH-r.1H-trans.10bH-cis.1-Acetylamino-8.9-methylenedioxy-1.2.4a.10b-tetrahydrodibenzo[b,d]pyrone-6 (10): A mixture of acetylamino bromolactone 9 (456.1 g, 1.163 mol) and DBU (182 g, 1.2 mol) in pyridine (5.0 L) is kept at reflux for 8½ hours under anhydrous conditions. After cooling overnight a crystalline material precipitates. The solid is filtered off and slurried with water for 10 minutes, filtered, and air-dried. The filtrate is evaporated to dryness at oil vacuum pressure, and the solid residue is slurried in water for 10 minutes, filtered, air-dried, and combined with the previous precipitate. Yield: 339.0 g (93.7%); m.p. 270-272°, lit. 263-267°.

4aH-r.1H-trans.1-Hydroxy-8.9-methylenedioxy-1.4.4a.10b-tetrahydro-6(5H)-phenanthridone (11): A mixture of acetylamino lactone 10 (60.0 g, 0.193 mol) and ethanol (150 mL) is treated with a solution of NaOH (30 g) in water (150 mL), then the reaction is heated to 90-95°C, and kept at that temperature for 8 hours. A small amount of water is added periodically to dissolve a solid that separates. The reaction mixture is allowed to cool to room temperature overnight, the precipitated solid is collected by filtration, washed with water and dried in air. The filtrate is acidified with concentrated hydrochloric acid, the resulting precipitate is filtered, washed with water (3 x 100 mL) and dried in air. Both precipitated solids are found to be identical therefore they are combined, however, NMR-analysis indicates the presence of substantial amounts of starting material. The product is redissolved in 20% sodium hydroxide (300 mL), kept at 120° for five hours, cooled, and reprecipitated. Repeating the procedure five times the obtained product does not show any more contamination. Yield: 94.0 g; m.p. 260-280° (dec.), lit. 265-280° (dec.).

4aH-r.1H-trans.1-(2'-Tetrahydropyranyloxy)-8.9-methylenedioxy-1.4.4a.10b-tetrahydro-6(5H)phenanthridone (12): To a suspension of hydroxyphenanthridone 11 (50 g, 0.193 mol) in dichloromethane (1.5 L) is added dihydropyran (70 mL, 0.857 mol) and p-toluene sulfonic acid (8.0 g). The reaction mixture is stirred for 72 hours with intermittent warming to 35°. The undissolved starting material is removed by filtration and the filtrate is washed with saturated sodium bicarbonate solution (2 x 500 mL) and water (500 mL). The organic layer is dried with sodium sulfate and evaporated to obtain a solid. Ethanol (25 mL) and ether (500 mL) is added to the solid and kept overnight at room temperature. The solid is filtered, washed with ether (2 x 200 mL) and air-dried to yield a white crystalline material. Yield: 54.0 g; m.p. 218°.

4aH-r, 1H-trans, 1-(2'-Tetrahydropyranyloxy)-2,3-epoxy-8,9-methylenedioxy-1,4,4a,10b-tetrahydro-6(5H)phenanthridone (13): Phenanthridone derivative 12 (82.0 g, 0.239 mol) and 3-chloroperoxybenzoic acid (82.0 g, 0.478 mol) are dissolved in methylene chloride (1.4 L) and stirred for two days. The organic solution is washed with saturated sodium bicarbonate solution (2 x 600 mL) and water (2 x 500 mL), dried over sodium sulfate and concentrated under reduced pressure to a volume of approx. 75 mL. Ether (200 mL) is added and the resulting precipitate is collected by filtration and washed with ether (100 mL). Yield: 71.1 g; m.p. 243°, lit. 250°.

4H-r, 1H-trans, 2H-cis, 10bH-trans, 1-(2'-Tetrahydropyranyloxy)-2-hydroxy-8,9-methylenedioxy-1,2,4a,10b-tetrahydro-6(5H)-phenanthridone (14): To a suspension of diphenyldiselenide (25.3 g, 0.081 mol) in anhydrous ethanol (500 mL) is added sodium borohydride (6.5 g, 0.17 mol) in small portions, while controlling the temperature with an ice bath. To the clear solution epoxide 13 (54.0 g, 0.15 mol) is added in one portion, then the reaction mixture is maintained at reflux temperature for two hours. Subsequently, the volume of the reaction solution is reduced to 250 mL by evaporation under reduced pressure, tetrahydrofuran (750 mL) is added, and the temperature is adjusted to about 3° in an ice bath. Hydrogen peroxide (30 wt. %, 250 mL, 2.2 mol equiv.) is slowly added while stirring, during which time a white solid precipitates. The reaction mixture is heated to reflux temperature, and reflux is maintained for 7 hours during which time all the solid dissolves and the reaction mixture becomes dark in color. Upon cooling, water (2 L) is added and the mixture is extracted with ethyl acetate (3 x 700 mL). The organic phase is washed with water (2 x 500 mL), dried over sodium sulfate, and upon evaporation of the solvent a white solid is obtained, which is washed with ether (2 x 200 mL). Yield: 38.5 g; m.p. 230-235°; lit. 232°.

4aH-r, 1H-trans, 2H-cis, 3H-trans, 4H-trans, 10bH-trans, 1-(2'-Tetrahydropyranyloxy)-2,3,4-trihydroxy-8,9-methylenedioxy-1,2,3,4,4a,10b-hexahydro-6(5H)phenanthridone (15): To a solution of N-methylmorpholine-N-oxide (16.0 g, 0.137 mol) in t-butanol (50 mL), acetone (50 mL), and water (20 mL) osmium tetroxide (260 mg, 1 mmol) is added. To this reaction medium is added a solution of intermediate 14 (28.8 g, 80 mmol) in t-butanol (700 mL) over a 10 minute period, followed by continued stirring for 48 hours. Decolorizing carbon is added to the dark solution, and after stirring for 3 hours at room temperature the reaction mixture is filtered through a Celite bed. The obtained pale-yellow solution is evaporated to an oil under reduced pressure. The residue is triturated with ethanol (25 mL), and the resulting crystalline material is collected by filtration. The solid is suspended in water (100 mL), stirred for one hour and filtered. The off-white solid is washed with water, and dried in air to give trihydroxy compound 15. Yield: 20.0 g; m.p. 222-223°.

AVS 360 OH

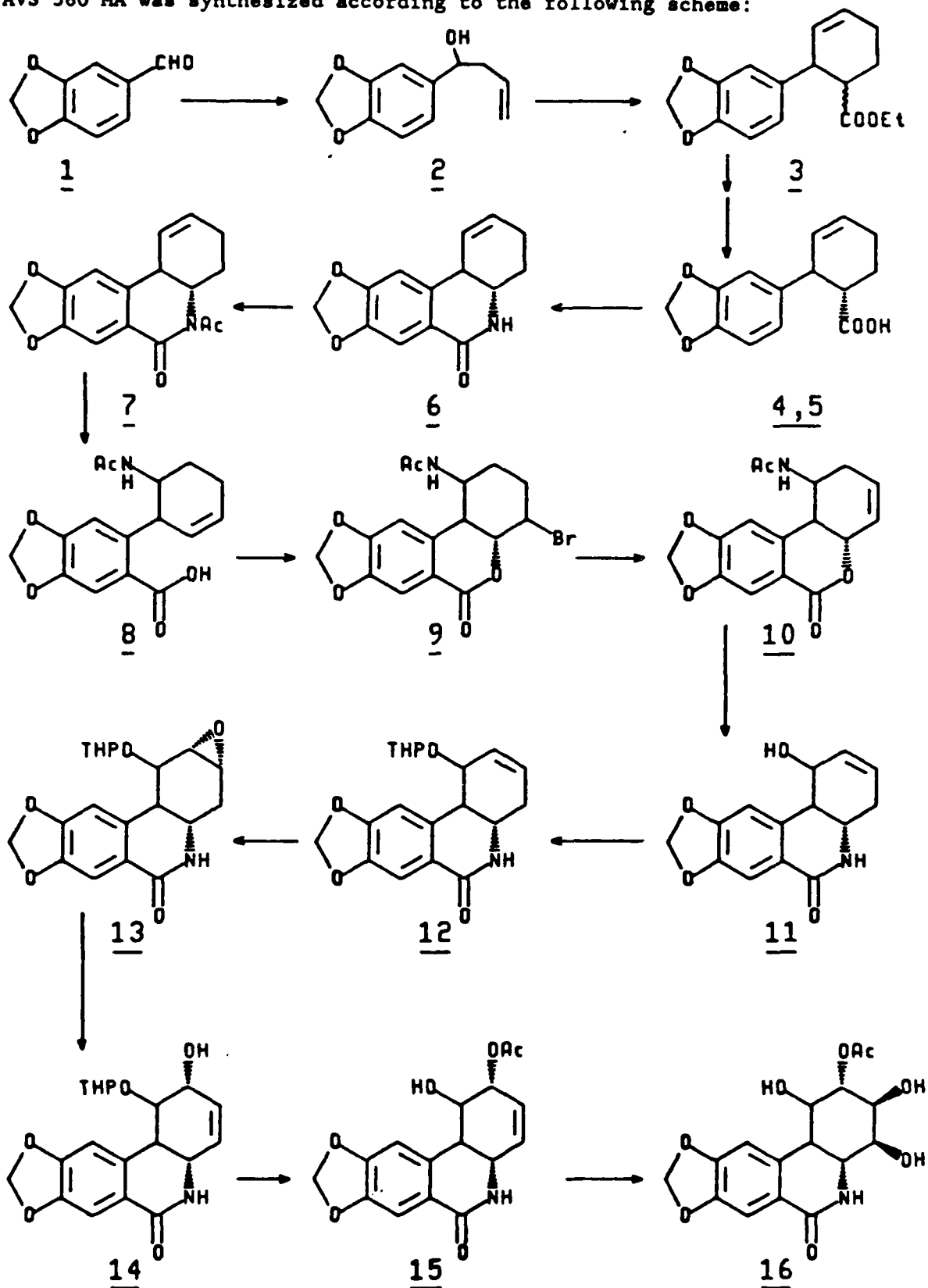
4aH-r.1H-trans.2H-cis.3H-trans.4H-trans.10bH-trans-1-Hydroxy-2,3,4-triacetoxy-8,9-methylenedioxy-1,2,3,4,4a,10b-hexahydro-6(5H)phenanthridone (16): A mixture of trihydroxy compound 15 (18.6 g, 0.047 mol), pyridine (200 mL) and acetic anhydride (200 mL) is stirred at room temperature overnight. Acetic anhydride and pyridine are evaporated under reduced pressure, followed by a distillation with ethanol to remove all pyridine. The residual material is soaked in ethanol (50 mL) and chilled. The crystalline material is collected by filtration, washed with ethanol (50 mL) and air-dried. Yield: 22.0 g; m.p. 275°C.

This material is suspended in ethanol (500 mL), p-toluene sulfonic acid (500 mg) is added, and the mixture is kept at reflux for 2 hours. The crystalline precipitate that forms is filtered off, washed with cold ethanol (2 x 25 mL) and dried in air. Yield: 15.0 g; m.p. 303-304°.

A small amount (0.8 g) is recrystallized from methanol (600 mL) to give the pure 16; yield: 500 mg.

M. 4aH-r, 1H-trans, 2H-cis, 3H-trans, 4H-trans, 1,3,4-Trihydroxy-2-acetoxy-8,9-methylenedioxy-1,2,3,4,4a,10b-hexahydro-6(5H)phenanthridone, AVS 360 MA

AVS 360 MA was synthesized according to the following scheme:



AVS360MA

Experimental^{10,11}

Ethyl-trans-2-(3',4'-methylenedioxyphenyl)-3-cyclohexene-1-carboxylic acid (3): Piperonal (500 g, 3.33 mol) is dissolved in tetrahydrofuran (3 L), and the solution is cooled below 5° in an ice bath. Allylmagnesium chloride solution (2000 mL, 4.0 mol) is added over a 2½ hour period while maintaining the temperature below 5°. After completion of addition, the reaction mixture is stirred an additional 30 minutes at low temperature. Then it is allowed to warm up to room temperature and kept there for three hours.

After cooling again below 5° a saturated ammonium chloride solution (1 L) is added during a 40 minute interval. The organic layer is separated and washed with brine (4 L) several times, and the aqueous layer is washed with chloroform (1 L). The combined organic layers are dried over sodium sulfate, filtered, and the solvent is removed under reduced pressure. Upon eliminating any remaining solvent under high vacuum, the resulting oil is applied in the subsequent reaction without further purification.

The obtained allyl carbinol derivative 2 (309 g) ethyl acrylate (195 g) and p-toluene sulfonic acid (54 g) are kept in a sealed bomb at 175-185° for six hours. Upon cooling the reaction mixture is submitted to distillation under reduced pressure to eliminate excess ethyl acrylate. The residue is dissolved in ether (3 L), the organic layer is washed with water (1 L), 5% sodium bicarbonate solution (1 L), and water (1 L). After drying with sodium sulfate overnight the solvent is taken off under reduced pressure and the residual oil is fractionally distilled where the desired product 3 boils at 145-155°/0.25 mm Hg, yield 263 g (90%). (Overall yield 29%)

trans-2-(3',4'-Methylenedioxyphenyl)-3-cyclohexene-1-carboxylic acid (4): A solution of sodium metal (10 g, 0.435 mol) in ethanol (300 mL) is combined with a solution of phenyl ester 3 in ethanol (300 mL), and the resulting mixture is kept at reflux for 2 hours. Water (60 mL) is added to the reaction mixture and reflux is continued for 4 more hours. The ethanol is evaporated under reduced pressure, the residue is dissolved in water (1 L) and washed with ether (3 x 300 mL). Upon cooling the aqueous layer, the pH is adjusted to pH 2 with conc. hydrochloric acid (prox. 25 mL), stirred for 30 minutes, and the precipitate is collected by filtration. The product is washed with water and air-dried to yield 81.1 g (90.3%) of acid 4; m.p. 101-102°, lit. 102-103°.

4aH-r,10bH-trans-8,9-Methylenedioxy-3,4,4a,10b-tetrahydro-6(5H)-phenanthridone (6): Carboxylic acid 4 (200 g, 0.81 mol) is dissolved in acetone (1 L), water (200 mL) and triethylamine (82 g, 0.81 mol) is added, and the mixture is cooled in an ice bath. A solution of ethyl chloroformate (87.4 g, 0.81 mol) in acetone (200 mL) is added slowly while stirring. Two hours after completion a solution of sodium azide (80 g, 1.23 mol) in water (200 mL) is added slowly during a one hour interval, followed by two hours of stirring at the lowered temperature. A mixture of toluene (1 L) and water (1.5 L) is added and stirring is continued for an additional 1.5 hours. The organic layer is separated, the aqueous layer washed with toluene (300 mL), and the combined organic layers are washed with water (2 x 1 L) and dried over sodium sulfate.

After filtering, the obtained solution is concentrated to 1700 mL under diminished pressure at 45°, followed by maintaining the concentrated solution at reflux for 4 hours. When TLC shows the disappearance of starting material the solution is cooled, and the solvent is evaporated under reduced pressure to leave 5 as a light tan colored oil, which is used in the next step without further purification.

The oily product 5 is cooled in an ice-water bath and carefully treated with boron trifluoride etherate (400 mL, 3.0 mol). The reaction mixture is allowed to stand at room temperature overnight. The crystalline solid that separates is filtered, washed with ether (3 x 200 mL) and dried in air; yield 155.0 g. The filtrate is evaporated to dryness under high vacuum, the residue is dissolved in ether (400 mL) and washed with ice-cold water (2 x 500 mL). The organic layer is dried over anhydrous sodium sulfate and evaporated to dryness. The obtained residue is again treated with boron trifluoride etherate (25 mL) and worked up as previously described to yield 4.4 g of additional product, which is identical to the main crop and hence they are combined. Total yield: 159.4 g (80.9%); m.p. 220-221° lit 220°.

4aH-r-10bH-trans-5-Acetyl-8,9-methylenedioxy-3,4,4a,10b-H-tetrahydro-6(5H)phenanthridone (7): A mixture of tetrahydrophenanthridone 6 (50.0 g, 0.206 mol), acetic anhydride (500 mL) and DMAP (1.0 g) is refluxed for 4 hours. Upon cooling a solid precipitates from solution. The precipitate is collected by filtration and sucked to dryness. The filtrate is evaporated under reduced pressure (40°), the resulting solid is triturated with ethanol, filtered, and the filter cake is combined with the one obtained previously. After an additional trituration of the combined product with ethanol, the solid is filtered off, washed with ice-cold ethanol and air dried. Yield: 40.0 g (62%); m.p. 155-157°, lit. 157-158°.

4H-r,3H-trans,3-(3',4'-Methylenedioxy-6'-carboxyphenyl)-4-acetamino-1-cyclohexene (8): N-acetylphenanthridone 7 (60.0 g, 0.2 mol) is suspended in methanol (1 L), then a solution of potassium hydroxide (22.4 g, 0.4 mol) in water (400 mL) is added, and the flask is immersed in a water bath preheated to 80°. The reaction mixture reaches a temperature of 70°C, and the reaction is kept at that temperature for 15 minutes. When cooled to room temperature a white solid starts to separate. The volume of the reaction mixture is reduced to 400 mL and the resulting precipitate is collected by filtration, washed with water (2 x 25 mL) and dried in air. It can be shown that the obtained precipitate is starting material 7.

The filtrate is cooled in an ice bath and acidified with con. hydrochloric acid to form a white precipitate that is filtered off, washed with water (2 x 25 mL) and dried in air. This product agrees in its spectral and analytical data with the structural assignment for 8, hence it is taken in the next step without further purification. Yield: 40.0 g (62%); m.p. 200-201°, lit. 198-201°. Recovered starting material: 20.5 g (37.0%).

4aH-r.1H-trans.10bH-cis.4H-trans.1-Acetylamino-4-bromo-8,9-methylenedioxy-1,2,3,4,4a,10b-hexahydrobenzo[b,d]pyrone-6 (9): To a well-stirred suspension of acetylamino carboxylic acid **8** (145.3 g, 0.48 mol) in tetrahydrofuran (2 L) powdered N-bromosuccinimide (89.0 g, 0.497 mol) is added in a single portion. Most of the solid dissolves, and a crystalline material begins to separate. After stirring for 1 hour the reaction mixture is cooled in an ice bath, then the precipitate is collected by filtration, washed with cold tetrahydrofurane, and dried. Yield: 175.5 g (93.3%); m.p. 265-267°, lit. 270°.

4aH-r.1H-trans.10bH-cis.1-Acetylamino-8,9-methylenedioxy-1,2,4a,10b-tetrahydridibenzo[b,d]pyrone-6 (10): A mixture of acetylamino bromolactone **9** (456.1 g, 1.163 mol) and DBU (182 g, 1.2 mol) in pyridine (5.0 L) is kept at reflux for 8½ hours under anhydrous conditions. After cooling overnight a crystalline material precipitates. The solid is filtered off and slurried with water for 10 minutes, filtered, and air-dried. The filtrate is evaporated to dryness at oil vacuum pressure, and the solid residue is slurried in water for 10 minutes, filtered, air-dried, and combined with the previous precipitate. Yield: 339.0 g (93.7%); m.p. 270-272°, lit. 263-267°.

4aH-r.1H-trans.1-Hydroxy-8,9-methylenedioxy-1,4,4a,10b-tetrahydro-6(5H)-phenanthridone (11): A mixture of acetylamino lactone **10** (60.0 g, 0.193 mol) and ethanol (150 mL) is treated with a solution of NaOH (30 g) in water (150 mL), then the reaction is heated to 90-95°C, and kept at that temperature for 8 hours. A small amount of water is added periodically to dissolve a solid that separates. The reaction mixture is allowed to cool to room temperature overnight, the precipitated solid is collected by filtration, washed with water and dried in air. The filtrate is acidified with concentrated hydrochloric acid, the resulting precipitate is filtered, washed with water (3 x 100 mL) and dried in air. Both precipitated solids are found to be identical therefore they are combined, however, NMR-analysis indicates the presence of substantial amounts of starting material. The product is redissolved in 20% sodium hydroxide (300 mL), kept at 120° for five hours, cooled, and reprecipitated. Repeating the procedure five times the obtained product does not show any more contamination. Yield: 94.0 g; m.p. 260-280° (dec.), lit. 265-280° (dec.).

4aH-r.1H-trans.1-(2'-Tetrahydropyranyloxy)-8,9-methylenedioxy-1,4,4a,10b-tetrahydro-6(5H)phenanthridone (12): To a suspension of hydroxyphenanthridone **11** (50 g, 0.193 mol) in dichloromethane (1.5 L) is added dihydropyran (70 mL, 0.857 mol) and p-toluene sulfonic acid (8.0 g). The reaction mixture is stirred for 72 hours with intermittent warming to 35°. The undissolved starting material is removed by filtration and the filtrate is washed with saturated sodium bicarbonate solution (2 x 500 mL) and water (500 mL). The organic layer is dried with sodium sulfate and evaporated to obtain a solid. Ethanol (25 mL) and ether (500 mL) are added to the solid and kept overnight at room temperature. The solid is filtered, washed with ether (2 x 200 mL) and air-dried to yield a white crystalline material. Yield: 54.0 g; m.p. 218°.

4aH-r.1H-trans.1-(2'-Tetrahydropyranyloxy)-2,3-epoxy-8,9-methylenedioxy-1,4,4a,10b-tetrahydro-6(5H)phenanthridone (13): Phenanthridone derivative 12 (82.0 g, 0.239 mol) and 3-chloroperoxybenzoic acid (82.0 g, 0.478 mol) are dissolved in methylene chloride (1.4 L) and stirred for two days. The organic solution is washed with saturated sodium bicarbonate solution (2 x 600 mL) and water (2 x 500 mL), dried over sodium sulfate and concentrated under reduced pressure to a volume of approx. 75 mL. Ether (200 mL) is added and the resulting precipitate is collected by filtration and washed with ether (100 mL). Yield: 71.1 g; m.p. 243°, lit. 250°.

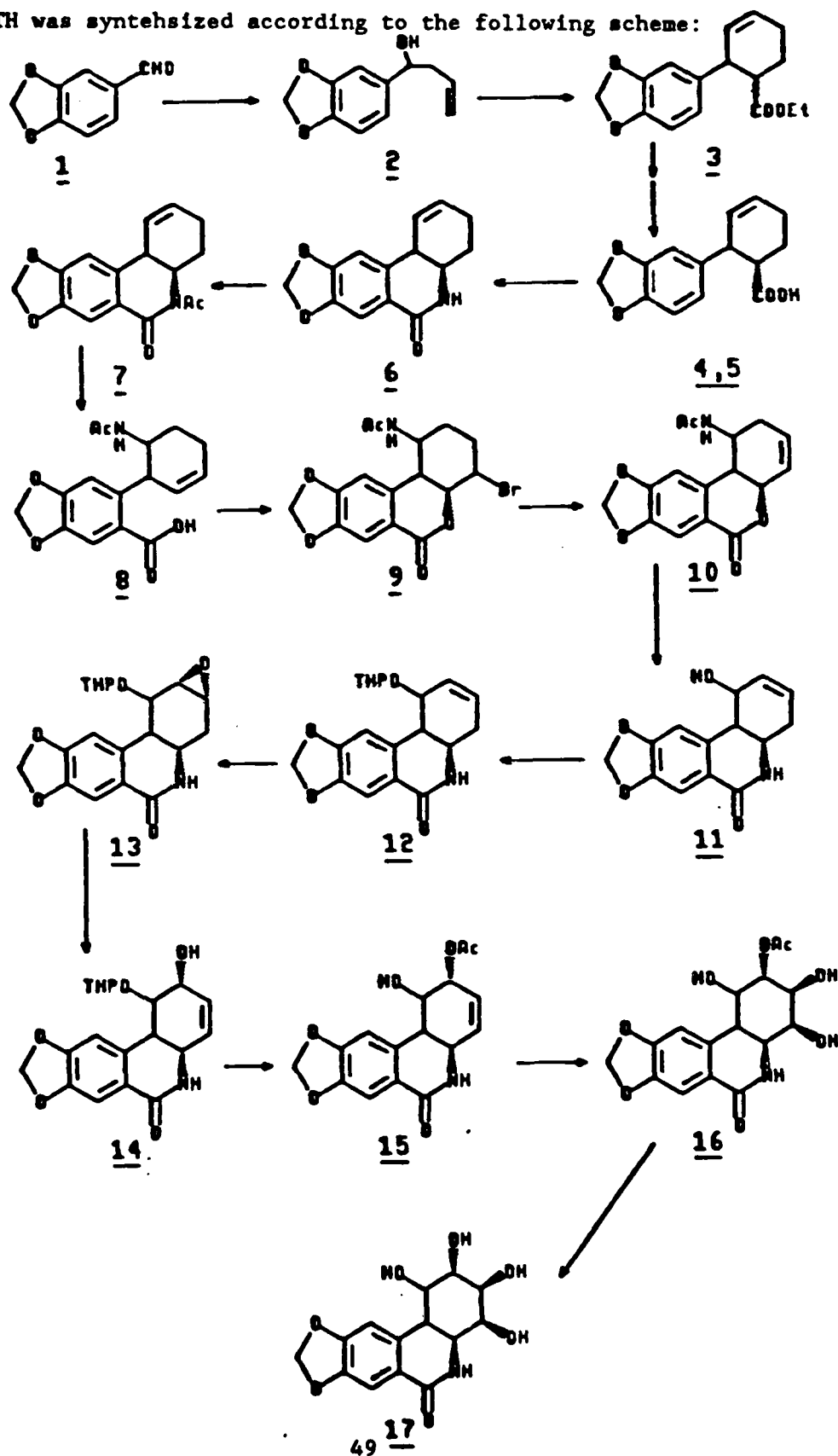
4H-r.1H-trans.2H-cis.10bH-trans.1-(2'-Tetrahydropyranyloxy)-2-hydroxy-8,9-methylenedioxy-1,2,4a,10b-tetrahydro-6(5H)-phenanthridone (14): To a suspension of diphenyldiselenide (25.3 g, 0.081 mol) in anhydrous ethanol (500 mL) is added sodium borohydride (6.5 g, 0.17 mol) in small portions, while controlling the temperature with an ice bath. To the clear solution epoxide 13 (54.0 g, 0.15 mol) is added in one portion, then the reaction mixture is maintained at reflux temperature for two hours. Subsequently, the volume of the reaction solution is reduced to 250 mL by evaporation under reduced pressure, tetrahydrofuran (750 mL) is added, and the temperature is adjusted to about 3° in an ice bath. Hydrogen peroxide (30 wt. %, 250 mL, 2.2 mol equiv.) is slowly added while stirring, during which time a white solid precipitates. The reaction mixture is heated to reflux temperature, and reflux is maintained for 7 hours during which time all the solid dissolves and the reaction mixture becomes dark in color. Upon cooling, water (2 L) is added and the mixture is extracted with ethyl acetate (3 x 700 mL). The organic phase is washed with water (2 x 500 mL), dried over sodium sulfate, and upon evaporation of the solvent a white solid is obtained, which is washed with ether (2 x 200 mL). Yield: 38.5 g; m.p. 230-235°; lit. 232°. The product (7 g) is recrystallized from ethanol to give 6.7 g pure product.

4aH-r.1H-trans.2H-cis.10bH-trans.1-Hydroxy-2-acetoxy-8,9-methylenedioxy-1,2,4a,10b-tetrahydro-6(5H)phenanthridone (15): A mixture of intermediate 14 (20.0 g, 55 mmol), acetic anhydride (400 mL), and dimethylaminopyridine (500 mg) are kept at reflux for 30 minutes. The excess acetic anhydride is evaporated under reduced pressure, and the residual acetic anhydride is coevaporated with ethanol (50 mL) twice. The obtained product is suspended in ethanol (400 mL), p-toluene-sulfonic acid (500 mg) is added and the mixture is kept at reflux for 3 hours. After cooling the product is collected by filtration, and upon concentrating the filtrate a second precipitate is obtained. Yield: 10.0 g (56.6%) m.p. 254° (dec.)

4aH-r.1H-trans.2H-cis.3H-trans.4H-trans.10bH-trans.1,3,4-Trihydroxy-2-acetoxy-8,9-methylenedioxy-1,2,3,4,4a,10b-hexahydro-6(5H)phenanthridone (16): A solution of intermediate 15 (9.8 g, 31 mmol) in acetone (900 mL) and t-butanol (300 mL) is combined with a solution of osmium tetroxide (200 mg) and N-methylmorpholine N-oxide (7.2 g, 61 mmol) in acetone (40 mL), t-butanol (40 mL) and water (20 mL). After 25 hours additional N-methylmorpholine N-oxide (3.0 g, 2.5 mmol) and osmium tetroxide (50 mg) are added, and the mixture is left over a three-day period. The solvent is evaporated under reduced pressure below 35°, and the residue is purified on a silica gel column with dichloromethane-methanol 9:1 (6 L) as the eluant. The combined fractions containing monoacetyl product 16 are combined, the volume is reduced to ca. 50 mL, and the resulting crystalline precipitate is collected by filtration to give 1.51 g (14%) of compound 16, m.p. 277° (decomp.).

N. 4aH-r,1H-trans,2H-cis,3H-trans,4H-trans,10bH-trans,1,2,3,4-Tetrahydroxy-
8,9-methylenedioxy-1,2,3,4,4a,10b-hexahydro-6(5H)phenanthridone, AVS 360 TH

AVS 360 TH was synthesized according to the following scheme:



Experimental^{10,11}

Ethyl-trans-2-(3',4'-methylenedioxyphenyl)-3-cyclohexene-1-carboxylic acid (3): Piperonal (500 g, 3.33 mol) is dissolved in tetrahydrofuran (3 L), and the solution is cooled below 5° in an ice bath. Allylmagnesium chloride solution (2000 mL, 4.0 mol) is added over a 2½ hour period while maintaining the temperature below 5°. After completion of addition, the reaction mixture is stirred an additional 30 minutes at low temperature. Then it is allowed to warm up to room temperature and kept there for three hours.

After cooling again below 5° a saturated ammonium chloride solution (1 L) is added during a 40 minute interval. The organic layer is separated and washed with brine (4 L) several times, and the aqueous layer is washed with chloroform (1 L). The combined organic layers are dried over sodium sulfate, filtered, and the solvent is removed under reduced pressure. Upon eliminating any remaining solvent under high vacuum, the resulting oil is applied in the subsequent reaction without further purification.

The obtained allyl carbinol derivative 2 (309 g) ethyl acrylate (195 g) and p-toluene sulfonic acid (54 g) are kept in a sealed bomb at 175-185° for six hours. Upon cooling the reaction mixture is submitted to distillation under reduced pressure to eliminate excess ethyl acrylate. The residue is dissolved in ether (3 L), the organic layer is washed with water (1 L), 5% sodium bicarbonate solution (1 L), and water (1 L). After drying with sodium sulfate overnight the solvent is taken off under reduced pressure and the residual oil is fractionally distilled where the desired product 3 boils at 145-155°/0.25 mm Hg, yield 263 g (90%). (Overall yield 29%)

trans-2-(3',4'-Methylenedioxyphenyl)-3-cyclohexene-1-carboxylic acid (4): A solution of sodium metal (10 g, 0.435 mol) in ethanol (300 mL) is combined with a solution of phenyl ester 3 in ethanol (300 mL), and the resulting mixture is kept at reflux for 2 hours. Water (60 mL) is added to the reaction mixture and reflux is continued for 4 more hours. The ethanol is evaporated under reduced pressure, the residue is dissolved in water (1 L) and washed with ether (3 x 300 mL). Upon cooling the aqueous layer, the pH is adjusted to pH 2 with conc. hydrochloric acid (prox. 25 mL), stirred for 30 minutes, and the precipitate is collected by filtration. The product is washed with water and air-dried to yield 81.1 g (90.3%) of acid 4; m.p. 101-102°, lit. 102-103°.

4aH-r.10bH-trans-8.9-Methylenedioxy-3.4.4a.10b-tetrahydro-6(5H)-phenanthridone (6): Carboxylic acid 4 (200 g, 0.81 mol) is dissolved in acetone (1 L), water (200 mL) and triethylamine (82 g, 0.81 mol) is added, and the mixture is cooled in an ice bath. A solution of ethyl chloroformate (87.4 g, 0.81 mol) in acetone (200 mL) is added slowly while stirring. Two hours after completion a solution of sodium azide (80 g, 1.23 mol) in water (200 mL) is added slowly during a one hour interval, followed by two hours of stirring at the lowered temperature. A mixture of toluene (1 L) and water (1.5 L) is added and stirring is continued for an additional 1.5 hours. The organic layer is separated, the aqueous layer washed with toluene (300 mL), and the combined organic layers are washed with water (2 x 1 L) and dried over sodium sulfate.

After filtering, the obtained solution is concentrated to 1700 mL under diminished pressure at 45°, followed by maintaining the concentrated solution at reflux for 4 hours. When TLC shows the disappearance of starting material the solution is cooled, and the solvent is evaporated under reduced pressure to leave 5 as a light tan colored oil, which is used in the next step without further purification.

The oily product 5 is cooled in an ice-water bath and carefully treated with boron trifluoride etherate (400 mL, 3.0 mol). The reaction mixture is allowed to stand at room temperature overnight. The crystalline solid that separates is filtered, washed with ether (3 x 200 mL) and dried in air; yield 155.0 g. The filtrate is evaporated to dryness under high vacuum, the residue is dissolved in ether (400 mL) and washed with ice-cold water (2 x 500 mL). The organic layer is dried over anhydrous sodium sulfate and evaporated to dryness. The obtained residue is again treated with boron trifluoride etherate (25 mL) and worked up as previously described to yield 4.4 g of additional product, which is identical to the main crop and hence they are combined. Total yield: 159.4 g (80.9%); m.p. 220-221° lit 220°.

4aH-r-10bH-trans-5-Acetyl-8,9-methylenedioxy-3,4,4a,10b-H-tetrahydro-6(5H)phenanthridone (7): A mixture of tetrahydrophenanthridone 6 (50.0 g, 0.206 mol), acetic anhydride (500 mL) and DMAP (1.0 g) is refluxed for 4 hours. Upon cooling a solid precipitates from solution. The precipitate is collected by filtration and sucked to dryness. The filtrate is evaporated under reduced pressure (40°), the resulting solid is triturated with ethanol, filtered, and the filter cake is combined with the one obtained previously. After an additional trituration of the combined product with ethanol, the solid is filtered off, washed with ice-cold ethanol and air dried. Yield: 40.0 g (62%); m.p. 155-157°, lit. 157-158°.

4H-r,3H-trans,3-(3',4'-Methylenedioxy-6'-carboxyphenyl)-4-acetamino-1-cyclohexene (8): N-acetylphenanthridone 7 (60.0 g, 0.2 mol) is suspended in methanol (1 L), then a solution of potassium hydroxide (22.4 g, 0.4 mol) in water (400 mL) is added, and the flask is immersed in a water bath preheated to 80°. The reaction mixture reaches a temperature of 70°C, and the reaction is kept at that temperature for 15 minutes. When cooled to room temperature a white solid starts to separate. The volume of the reaction mixture is reduced to 400 mL and the resulting precipitate is collected by filtration, washed with water (2 x 25 mL) and dried in air. It can be shown that the obtained precipitate is starting material 7.

The filtrate is cooled in an ice bath and acidified with con. hydrochloric acid to form a white precipitate that is filtered off, washed with water (2 x 25 mL) and dried in air. This product agrees in its spectral and analytical data with the structural assignment for 8, hence it is taken in the next step without further purification. Yield: 40.0 g (62%); m.p. 200-201°, lit. 198-201°. Recovered starting material: 20.5 g (37.0%).

4aH-r,1H-trans,10bH-cis,4H-trans,1-Acetylamino-4-bromo-8,9-methylene-dioxy-1,2,3,4,4a,10b-hexahydrobenzo[b,d]pyrone-6 (9): To a well-stirred suspension of acetylamino carboxylic acid 8 (145.3 g, 0.48 mol) in tetrahydrofuran (2 L) powdered N-bromosuccinimide (89.0 g, 0.497 mol) is added in a single portion. Most of the solid dissolves, and a crystalline material begins to separate. After stirring for 1 hour the reaction mixture is cooled in an ice bath, then the precipitate is collected by filtration, washed with cold tetrahydrofurane, and dried. Yield: 175.5 g (93.3%); m.p. 265-267°, lit. 270°.

4aH-r,1H-trans,10bH-cis,1-Acetylamino-8,9-methylenedioxy-1,2,4a,10b-tetrahydrodibenzo[b,d]pyrone-6 (10): A mixture of acetylaminobromolactone 9 (456.1 g, 1.163 mol) and DBU (182 g, 1.2 mol) in pyridine (5.0 L) is kept at reflux for 8½ hours under anhydrous conditions. After cooling overnight a crystalline material precipitates. The solid is filtered off and slurried with water for 10 minutes, filtered, and air-dried. The filtrate is evaporated to dryness at oil vacuum pressure, and the solid residue is slurried in water for 10 minutes, filtered, air-dried, and combined with the previous precipitate. Yield: 339.0 g (93.7%); m.p. 270-272°, lit. 263-267°.

4aH-r,1H-trans,1-Hydroxy-8,9-methylenedioxy-1,4,4a,10b-tetrahydro-6(5H)-phenanthridone (11): A mixture of acetylaminolactone 10 (60.0 g, 0.193 mol) and ethanol (150 mL) is treated with a solution of NaOH (30 g) in water (150 mL), then the reaction is heated to 90-95°C, and kept at that temperature for 8 hours. A small amount of water is added periodically to dissolve a solid that separates. The reaction mixture is allowed to cool to room temperature overnight, the precipitated solid is collected by filtration, washed with water and dried in air. The filtrate is acidified with concentrated hydrochloric acid, the resulting precipitate is filtered, washed with water (3 x 100 mL) and dried in air. Both precipitated solids are found to be identical therefore they are combined, however, NMR-analysis indicates the presence of substantial amounts of starting material. The product is redissolved in 20% sodium hydroxide (300 mL), kept at 120° for five hours, cooled, and reprecipitated. Repeating the procedure five times the obtained product does not show any more contamination. Yield: 94.0 g; m.p. 260-280° (dec.), lit. 265-280° (dec.).

4aH-r,1H-trans,1-(2'-Tetrahydropyranyloxy)-8,9-methylenedioxy-1,4,4a,10b-tetrahydro-6(5H)phenanthridone (12): To a suspension of hydroxy-phenanthridone 11 (50 g, 0.193 mol) in dichloromethane (1.5 L) is added dihydropyran (70 mL, 0.857 mol) and p-toluene sulfonic acid (8.0 g). The reaction mixture is stirred for 72 hours with intermittent warming to 35°. The undissolved starting material is removed by filtration and the filtrate is washed with saturated sodium bicarbonate solution (2 x 500 mL) and water (500 mL). The organic layer is dried with sodium sulfate and evaporated to obtain a solid. Ethanol (25 mL) and ether (500 mL) are added to the solid and kept overnight at room temperature. The solid is filtered, washed with ether (2 x 200 mL) and air-dried to yield a white crystalline material. Yield: 54.0 g; m.p. 218°.

4aH-r,1H-trans,1-(2'-Tetrahydropyranyloxy)-2,3-epoxy-8,9-methylenedioxy-1,4,4a,10b-tetrahydro-6(5H)phenanthridone (13): Phenanthridone derivative 12 (82.0 g, 0.239 mol) and 3-chloroperoxybenzoic acid (82.0 g, 0.478 mol) are dissolved in methylene chloride (1.4 L) and stirred for two days. The organic solution is washed with saturated sodium bicarbonate solution (2 x 600 mL) and water (2 x 500 mL), dried over sodium sulfate and concentrated under reduced pressure to a volume of approx. 75 mL. Ether (200 mL) is added and the resulting precipitate is collected by filtration and washed with ether (100 mL). Yield: 71.1 g; m.p. 243°, lit. 250°.

4H-r,1H-trans,2H-cis,10bH-trans,1-(2'-Tetrahydropyranyloxy)-2-hydroxy-8,9-methylenedioxy-1,2,4a,10b-tetrahydro-6(5H)-phenanthridone (14): To a suspension of diphenyldiselenide (25.3 g, 0.081 mol) in anhydrous ethanol (500 mL) is added sodium borohydride (6.5 g, 0.17 mol) in small portions, while controlling the temperature with an ice bath. To the clear solution epoxide 13 (54.0 g, 0.15 mol) is added in one portion, then the reaction mixture is maintained at reflux temperature for two hours. Subsequently, the volume of the reaction solution is reduced to 250 mL by evaporation under reduced pressure, tetrahydrofuran (750 mL) is added, and the temperature is adjusted to about 3° in an ice bath. Hydrogen peroxide (30 wt. %, 250 mL, 2.2 mol equiv.) is slowly added while stirring, during which time a white solid precipitates. The reaction mixture is heated to reflux temperature, and reflux is maintained for 7 hours during which time all the solid dissolves and the reaction mixture becomes dark in color. Upon cooling, water (2 L) is added and the mixture is extracted with ethyl acetate (3 x 700 mL). The organic phase is washed with water (2 x 500 mL), dried over sodium sulfate, and upon evaporation of the solvent a white solid is obtained, which is washed with ether (2 x 200 mL). Yield: 38.5 g; m.p. 230-235°; lit. 232°.

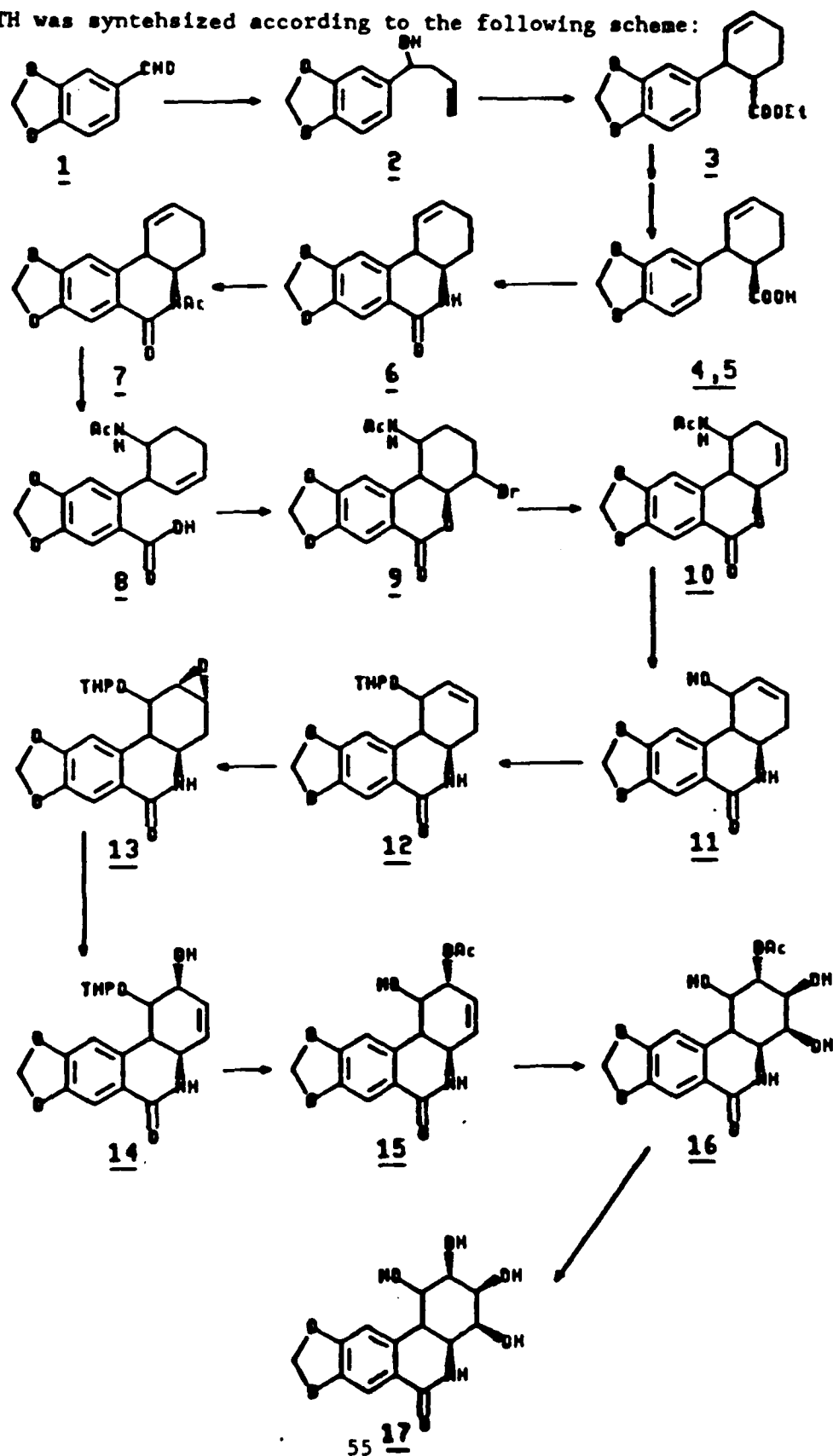
The product (7 g) is recrystallized from ethanol to give 6.7 g pure product.

4aH-r,1H-trans,2H-cis,3H-trans,4H-trans,10bH-trans,1-(2'-tetrahydropyranyloxy)-2,3,4-trihydroxy-8,9-methylenedioxy-1,2,3,4,4a,10b-hexahydro-6(5H)phenanthridone (15): Osmium tetroxide (100 mg) is added to a solution of methylmorpholine-N-oxide (2.2 g) dissolved in t-butanol (25 mL), acetone (25 mL) and water (20 mL), and the mixture is stirred for 10 minutes. A warm solution of phenanthridone 14 (4.0 g, 11 mmol) in t-butanol (200 mL) and acetone (200 mL) is added to the osmium tetroxide solution over a five minute period. TLC indicates that after 24 hours the reaction shows about 60% conversion, therefore 500 mg N-methylmorpholine-N-oxide and 50 mg of osmiumtetroxide are added. After another 24-hour period and 95% conversion the reaction is terminated by evaporating the solvent under reduced pressure at 35°C bath temperature. The resulting tarry residue is triturated with ethanol (25 mL), the ethanol is evaporated, another trituration with ethanol (25 mL) leaves the product as a crystalline suspension in ethanol, and upon filtration product 15 is obtained as a pale-yellow solid; yield 3.6 g (82%), m.p. 222-224°. This material is used in the next step without further purification.

4aH-r, 1H-trans, 2H-cis, 3H-trans, 4H-trans, 10bH-trans, 1,2,3,4-tetrahydroxy-8,9-methylenedioxy-1,2,3,4,4a,10b-hexahydro-6(5H)-phenanthridone (16): Trihydroxy compound 15 (3.4 g, 8.6 mmol) is suspended in ethanol (150 mL), and p-toluene sulfonic acid (200 mg) is added. The reaction mixture is heated to reflux, and TLC (Silica gel; chloroform-methanol 6:1) indicates that the reaction reaches completion after 3 hours at reflux. During reflux the reaction mixture turns clear after about 1½ hours and a product starts to crystallize. Upon cooling the precipitated crystals are collected by filtration, the mother liquor produces a second crop after concentration and the combined precipitates are recrystallized from acetic acid (250 mL). Since the obtained material shows impurities, a second recrystallization from water produces tetrahydroxy compound 16 as an analytically pure, off-white amorphous powder. Yield: 57%, m.p. 308° (dec.); lit.

0. 4aH-r,1H-trans,2H-cis,3H-trans,4H-trans,10bH-trans,1,2,3,4-Tetrahydroxy-8,9-methylenedioxy-1,2,3,4,4a,10b-hexahydro-6(5H)phenanthridone, AVS 360 TH

AVS 360 TH was synthesized according to the following scheme:



Experimental^{10,11}

Ethyl-trans-2-(3',4'-methylenedioxyphenyl)-3-cyclohexene-1-carboxylic acid (3): Piperonal (500 g, 3.33 mol) is dissolved in tetrahydrofuran (3 L) and the solution is cooled below 5° in an ice bath. Allylmagnesium chloride solution (2000 mL, 4.0 mol) is added over a 2½ hour period while maintaining the temperature below 5°. After completion of addition, the reaction mixture is stirred an additional 30 minutes at low temperature. Then it is allowed to warm up to room temperature and kept there for three hours.

After cooling again below 5° a saturated ammonium chloride solution (1 L) is added during a 40 minute interval. The organic layer is separated and washed with brine (4 L) several times, and the aqueous layer is washed with chloroform (1 L). The combined organic layers are dried over sodium sulfate, filtered, and the solvent is removed under reduced pressure. Upon eliminating any remaining solvent under high vacuum, the resulting oil is applied in the subsequent reaction without further purification.

The obtained allyl carbinol derivative 2 (309 g) ethyl acrylate (195 g) and p-toluene sulfonic acid (54 g) are kept in a sealed bomb at 175-185° for six hours. Upon cooling the reaction mixture is submitted to distillation under reduced pressure to eliminate excess ethyl acrylate. The residue is dissolved in ether (3 L), the organic layer is washed with water (1 L), 5% sodium bicarbonate solution (1 L), and water (1 L). After drying with sodium sulfate overnight the solvent is taken off under reduced pressure and the residual oil is fractionally distilled where the desired product 3 boils at 145-155°/0.25 mm Hg, yield 263 g (90%). (Overall yield 29%).

trans-2-(3',4'-Methylenedioxyphenyl)-3-cyclohexene-1-carboxylic acid (4): A solution of sodium metal (10 g, 0.435 mol) in ethanol (300 mL) is combined with a solution of phenyl ester 3 in ethanol (300 mL), and the resulting mixture is kept at reflux for 2 hours. Water (60 mL) is added to the reaction mixture and reflux is continued for 4 more hours. The ethanol is evaporated under reduced pressure, the residue is dissolved in water (1 L) and washed with ether (3 x 300 mL). Upon cooling the aqueous layer, the pH is adjusted to pH 2 with conc. hydrochloric acid (prox. 25 mL), stirred for 30 minutes, and the precipitate is collected by filtration. The product is washed with water and air-dried to yield 81.1 g (90.3%) of acid 4; m.p. 101-102°, lit. 102-103°.

4aH-r,10bH-trans-8,9-Methylenedioxy-3,4,4a,10b-tetrahydro-6(5H)phenanthridone (6): Carboxylic acid 4 (200 g, 0.81 mol) is dissolved in acetone (1 L), water (200 mL) and triethylamine (82 g, 0.81 mol) is added, and the mixture is cooled in an ice bath. A solution of ethyl chloroformate (87.4 g, 0.81 mol) in acetone (200 mL) is added slowly while stirring. Two hours after completion a solution of sodium azide (80 g, 1.23 mol) in water (200 mL) is added slowly during a one hour interval, followed by two hours of stirring at the lowered temperature. A mixture of toluene (1 L) and water (1.5 L) is added and stirring is continued for an additional 1.5 hours. The organic layer is separated, the aqueous layer washed with toluene (300 mL), and the combined organic layers are washed with water (2 x 1 L) and dried over sodium sulfate.

After filtering, the obtained solution is concentrated to 1700 mL under diminished pressure at 45°, followed by maintaining the concentrated solution at reflux for 4 hours. When TLC shows the disappearance of starting material the solution is cooled, and the solvent is evaporated under reduced pressure to leave 5 as a light tan colored oil, which is used in the next step without further purification.

The oily product 5 is cooled in an ice-water bath and carefully treated with boron trifluoride etherate (400 mL, 3.0 mol). The reaction mixture is allowed to stand at room temperature overnight. The crystalline solid that separates is filtered, washed with ether (3 x 200 mL) and dried in air; yield 155.0 g. The filtrate is evaporated to dryness under high vacuum, the residue is dissolved in ether (400 mL) and washed with ice-cold water (2 x 500 mL). The organic layer is dried over anhydrous sodium sulfate and evaporated to dryness. The obtained residue is again treated with boron trifluoride etherate (25 mL) and worked up as previously described to yield 4.4 g of additional product, which is identical to the main crop and hence they are combined. Total yield: 159.4 g (80.9%); m.p. 220-221° lit 220°.

4aH-r-10bH-trans-5-Acetyl-8,9-methylenedioxy-3,4,4a,10b-H-tetrahydro-6(5H)phenanthridone (7): A mixture of tetrahydrophenanthridone 6 (50.0 g, 0.206 mol), acetic anhydride (500 mL) and DMAP (1.0 g) is refluxed for 4 hours. Upon cooling a solid precipitates from solution. The precipitate is collected by filtration and sucked to dryness. The filtrate is evaporated under reduced pressure (40°), the resulting solid is triturated with ethanol, filtered, and the filter cake is combined with the one obtained previously. After an additional trituration of the combined product with ethanol, the solid is filtered off, washed with ice-cold ethanol and air dried. Yield: 40.0 g (62%); m.p. 155-157°, lit. 157-158°.

4H-r,3H-trans,3-(3',4'-Methylenedioxy-6'-carboxyphenyl)-4-acetamino-1-cyclohexene (8): N-acetylphenanthridone 7 (60.0 g, 0.2 mol) is suspended in methanol (1 L), then a solution of potassium hydroxide (22.4 g, 0.4 mol) in water (400 mL) is added, and the flask is immersed in a water bath preheated to 80°. The reaction mixture reaches a temperature of 70°C, and the reaction is kept at that temperature for 15 minutes. When cooled to room temperature a white solid starts to separate. The volume of the reaction mixture is reduced to 400 mL and the resulting precipitate is collected by filtration, washed with water (2 x 25 mL) and dried in air. It can be shown that the obtained precipitate is starting material 7.

The filtrate is cooled in an ice bath and acidified with con. hydrochloric acid to form a white precipitate that is filtered off, washed with water (2 x 25 mL) and dried in air. This product agrees in its spectral and analytical data with the structural assignment for 8, hence it is taken in the next step without further purification. Yield: 40.0 g (62%); m.p. 200-201°, lit. 198-201°. Recovered starting material: 20.5 g (37.0%).

4aH-r, 1H-trans, 10bH-cis, 4H-trans, 1-Acetylamino-4-bromo-8,9-methylenedioxy-1,2,3,4,4a,10b-hexahydrobenzo[b,d]pyrone-6 (9): To a well-stirred suspension of acetylamino carboxylic acid **8** (145.3 g, 0.48 mol) in tetrahydrofuran (2 L) powdered N-bromosuccinimide (89.0 g, 0.497 mol) is added in a single portion. Most of the solid dissolves, and a crystalline material begins to separate. After stirring for 1 hour the reaction mixture is cooled in an ice bath, then the precipitate is collected by filtration, washed with cold tetrahydrofurane, and dried. Yield: 175.5 g (93.3%); m.p. 265-267°, lit. 270°.

4aH-r, 1H-trans, 10bH-cis, 1-Acetylamino-8,9-methylenedioxy-1,2,4a,10b-tetrahydridibenzo[b,d]pyrone-6 (10): A mixture of acetylaminobromolactone **2** (456.1 g, 1.163 mol) and DBU (182 g, 1.2 mol) in pyridine (5.0 L) is kept at reflux for 8½ hours under anhydrous conditions. After cooling overnight a crystalline material precipitates. The solid is filtered off and slurred with water for 10 minutes, filtered, and air-dried. The filtrate is evaporated to dryness at oil vacuum pressure, and the solid residue is slurred in water for 10 minutes, filtered, air-dried, and combined with the previous precipitate. Yield: 339.0 g (93.7%); m.p. 270-272°, lit. 263-267°.

4aH-r, 1H-trans, 1-Hydroxy-8,9-methylenedioxy-1,4,4a,10b-tetrahydro-6(5H)-phenanthridone (11): A mixture of acetylaminolactone **10** (60.0 g, 0.193 mol) and ethanol (150 mL) is treated with a solution of NaOH (30 g) in water (150 mL), then the reaction is heated to 90-95°C, and kept at that temperature for 8 hours. A small amount of water is added periodically to dissolve a solid that separates. The reaction mixture is allowed to cool to room temperature overnight, the precipitated solid is collected by filtration, washed with water and dried in air. The filtrate is acidified with concentrated hydrochloric acid, the resulting precipitate is filtered, washed with water (3 x 100 mL) and dried in air. Both precipitated solids are found to be identical therefore they are combined, however, NMR-analysis indicates the presence of substantial amounts of starting material. The product is redissolved in 20% sodium hydroxide (300 mL), kept at 120° for five hours, cooled, and reprecipitated. Repeating the procedure five times the obtained product does not show any more contamination. Yield: 94.0 g; m.p. 260-280° (dec.), lit. 265-280° (dec.).

4aH-r, 1H-trans, 1-(2'-Tetrahydropyranyloxy)-8,9-methylenedioxy-1,4,4a,10b-tetrahydro-6(5H)phenanthridone (12): To a suspension of hydroxyphenanthridone **11** (50 g, 0.193 mol) in dichloromethane (1.5 L) is added dihydropyran (70 mL, 0.857 mol) and p-toluene sulfonic acid (8.0 g). The reaction mixture is stirred for 72 hours with intermittent warming to 35°. The undissolved starting material is removed by filtration and the filtrate is washed with saturated sodium bicarbonate solution (2 x 500 mL) and water (500 mL). The organic layer is dried with sodium sulfate and evaporated to obtain a solid. Ethanol (25 mL) and ether (500 mL) are added to the solid and kept overnight at room temperature. The solid is filtered, washed with ether (2 x 200 mL) and air-dried to yield a white crystalline material. Yield: 54.0 g; m.p. 218°.

4aH-r.1H-trans.1-(2'-Tetrahydropyranyloxy)-2,3-epoxy-8,9-methylenedioxy-1,4,4a,10b-tetrahydro-6(5H)phenanthridone (13): Phenanthridone derivative 12 (82.0 g, 0.239 mol) and 3-chloroperoxybenzoic acid (82.0 g, 0.478 mol) are dissolved in methylene chloride (1.4 L) and stirred for two days. The organic solution is washed with saturated sodium bicarbonate solution (2 x 600 mL) and water (2 x 500 mL), dried over sodium sulfate and concentrated under reduced pressure to a volume of approx. 75 mL. Ether (200 mL) is added and the resulting precipitate is collected by filtration and washed with ether (100 mL). Yield: 71.1 g; m.p. 243°, lit. 250°.

4H-r.1H-trans.2H-cis.10bH-trans.1-(2'-Tetrahydropyranyloxy)-2-hydroxy-8,9-methylenedioxy-1,2,4a,10b-tetrahydro-6(5H)-phenanthridone (14): To a suspension of diphenyldiselenide (25.3 g, 0.081 mol) in anhydrous ethanol (500 mL) is added sodium borohydride (6.5 g, 0.17 mol) in small portions, while controlling the temperature with an ice bath. To the clear solution epoxide 13 (54.0 g, 0.15 mol) is added in one portion, then the reaction mixture is maintained at reflux temperature for two hours. Subsequently, the volume of the reaction solution is reduced to 250 mL by evaporation under reduced pressure, tetrahydrofuran (750 mL) is added, and the temperature is adjusted to about 3° in an ice bath. Hydrogen peroxide (30 wt. %, 250 mL, 2.2 mol equiv.) is slowly added while stirring, during which time a white solid precipitates. The reaction mixture is heated to reflux temperature, and reflux is maintained for 7 hours during which time all the solid dissolves and the reaction mixture becomes dark in color. Upon cooling, water (2 L) is added and the mixture is extracted with ethyl acetate (3 x 700 mL). The organic phase is washed with water (2 x 500 mL), dried over sodium sulfate, and upon evaporation of the solvent a white solid is obtained, which is washed with ether (2 x 200 mL). Yield: 38.5 g; m.p. 230-235°; lit. 232°. The product (7 g) is recrystallized from ethanol to give 6.7 g pure product.

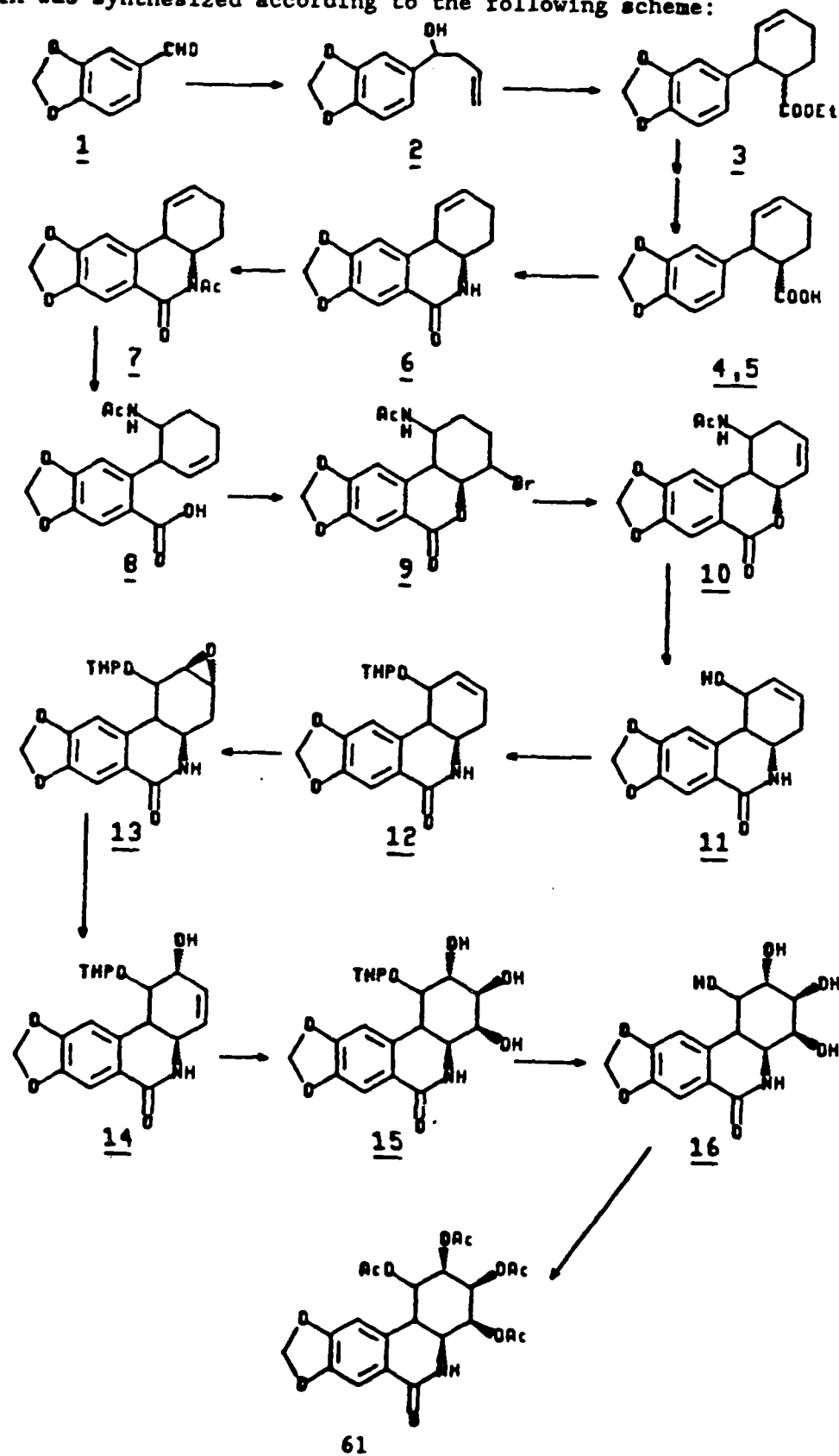
4aH-r.1H-trans.2H-cis.3H-trans.4H-trans.10bH-trans.1,3,4-hydroxy-2-acetoxy-8,9-methylenedioxy-1,2,3,4,4a,10b-hexahydro-6(5H)phenanthridone (16): Osmium tetroxide (100 mg) is added to a solution of methylmorpholine-N-oxide (2.2 g) dissolved in t-butanol (25 mL), acetone (25 mL) and water (20 mL), and the mixture is stirred for 10 minutes. A warm solution of phenanthridone 14 (4.0 g, 11 mmol) in t-butanol (200 mL) and acetone (200 mL) is added to the osmium tetroxide solution over a five minute period. TLC indicates that after 24 hours the reaction shows about 60% conversion, therefore 500 mg N-methylmorpholine-N-oxide and 50 mg of osmiumtetroxide are added. After another 24-hour period and 95% conversion the reaction is terminated by evaporating the solvent under reduced pressure at 35°C bath temperature. The resulting tarry residue is triturated with ethanol (25 mL), the ethanol is evaporated, another trituration with ethanol (25 mL) leaves the product as a crystalline suspension in ethanol, and upon filtration product 15 is obtained as a pale-yellow solid; yield 3.6 g (82%), m.p. 222-224°. This material is used in the next step without further purification.

AVS 360 TH

4aH-r. 1H-trans. 2H-cis. 3H-trans. 4H-trans. 10bH-trans. 1.2.3.4-tetrahydroxy-8.9-methylenedioxy-1.2.3.4.4a.10b-hexahydro-6(5H)-phenanthridone (17): Trihydroxy compound 15 (3.4 g, 8.6 mmol) is suspended in ethanol (150 mL), and p-toluene sulfonic acid (200 mg) is added. The reaction mixture is heated to reflux, and TLC (Silica gel; chloroform-methanol 6:1) indicates that the reaction reaches completion after 3 hours at reflux. During reflux the reaction mixture turns clear after about 1½ hours and a product starts to crystallize. Upon cooling the precipitated crystals are collected by filtration, the mother liquor produces a second crop after concentration and the combined precipitates are recrystallized from acetic acid (250 mL). Since the obtained material shows impurities, a second recrystallization from water produces tetrahydroxy compound 16 as an analytically pure, off-white amorphous powder. Yield: 57%, m.p. 308° (dec.); lit.

P. 4aH-r, 1H-trans, 2H-cis, 3H-trans, 4H-trans, 10bH-trans, 1,2,3,4-Tetraacetoxy-
8,9-methylenedioxy-1,2,3,4,4a,10b-hexahydro-6(5H)phenanthridone, AVS 360 TA

AVS 360 TA was synthesized according to the following scheme:



Experimental^{10,11}

Ethyl-trans-2-(3',4'-methylenedioxyphenyl)-3-cyclohexene-1-carboxylic acid (3): Piperonal (500 g, 3.33 mol) is dissolved in tetrahydrofuran (3 L), and the solution is cooled below 5° in an ice bath. Allylmagnesium chloride solution (2000 mL, 4.0 mol) is added over a 2½ hour period while maintaining the temperature below 5°. After completion of addition, the reaction mixture is stirred an additional 30 minutes at low temperature. Then it is allowed to warm up to room temperature and kept there for three hours.

After cooling again below 5° a saturated ammonium chloride solution (1 L) is added during a 40 minute interval. The organic layer is separated and washed with brine (4 L) several times, and the aqueous layer is washed with chloroform (1 L). The combined organic layers are dried over sodium sulfate, filtered, and the solvent is removed under reduced pressure. Upon eliminating any remaining solvent under high vacuum, the resulting oil is applied in the subsequent reaction without further purification.

The obtained allyl carbinol derivative 2 (309 g) ethyl acrylate (195 g) and p-toluene sulfonic acid (54 g) are kept in a sealed bomb at 175-185° for six hours. Upon cooling the reaction mixture is submitted to distillation under reduced pressure to eliminate excess ethyl acrylate. The residue is dissolved in ether (3 L), the organic layer is washed with water (1 L), 5% sodium bicarbonate solution (1 L), and water (1 L). After drying with sodium sulfate overnight the solvent is taken off under reduced pressure and the residual oil is fractionally distilled where the desired product 3 boils at 145-155°/0.25 mm Hg, yield 263 g (90%). (Overall yield 29%).

trans-2-(3',4'-Methylenedioxyphenyl)-3-cyclohexene-1-carboxylic acid (4): A solution of sodium metal (10 g, 0.435 mol) in ethanol (300 mL) is combined with a solution of phenyl ester 3 in ethanol (300 mL), and the resulting mixture is kept at reflux for 2 hours. Water (60 mL) is added to the reaction mixture and reflux is continued for 4 more hours. The ethanol is evaporated under reduced pressure, the residue is dissolved in water (1 L) and washed with ether (3 x 300 mL). Upon cooling the aqueous layer, the pH is adjusted to pH 2 with conc. hydrochloric acid (prox. 25 mL), stirred for 30 minutes, and the precipitate is collected by filtration. The product is washed with water and air-dried to yield 81.1 g (90.3%) of acid 4; m.p. 101-102°, lit. 102-103°.

4aH-r.10bH-trans-8,9-Methylenedioxy-3,4,4a,10b-tetrahydro-6(5H)-phenanthridone (6): Carboxylic acid 4 (200 g, 0.81 mol) is dissolved in acetone (1 L), water (200 mL) and triethylamine (82 g, 0.81 mol) is added, and the mixture is cooled in an ice bath. A solution of ethyl chloroformate (87.4 g, 0.81 mol) in acetone (200 mL) is added slowly while stirring. Two hours after completion a solution of sodium azide (80 g, 1.23 mol) in water (200 mL) is added slowly during a one hour interval, followed by two hours of stirring at the lowered temperature. A mixture of toluene (1 L) and water (1.5 L) is added and stirring is continued for an additional 1.5 hours. The organic layer is separated, the aqueous layer washed with toluene (300 mL), and the combined organic layers are washed with water (2 x 1 L) and dried over sodium sulfate.

After filtering, the obtained solution is concentrated to 1700 mL under diminished pressure at 45°, followed by maintaining the concentrated solution at reflux for 4 hours. When TLC shows the disappearance of starting material the solution is cooled, and the solvent is evaporated under reduced pressure to leave 5 as a light tan colored oil, which is used in the next step without further purification.

The oily product 5 is cooled in an ice-water bath and carefully treated with boron trifluoride etherate (400 mL, 3.0 mol). The reaction mixture is allowed to stand at room temperature overnight. The crystalline solid that separates is filtered, washed with ether (3 x 200 mL) and dried in air; yield 155.0 g. The filtrate is evaporated to dryness under high vacuum, the residue is dissolved in ether (400 mL) and washed with ice-cold water (2 x 500 mL). The organic layer is dried over anhydrous sodium sulfate and evaporated to dryness. The obtained residue is again treated with boron trifluoride etherate (25 mL) and worked up as previously described to yield 4.4 g of additional product, which is identical to the main crop and hence they are combined. Total yield: 159.4 g (80.9%); m.p. 220-221° lit 220°.

4aH-r-10bH-trans-5-Acetyl-8,9-methylenedioxy-3,4,4a,10b-H-tetrahydro-6(5H)phenanthridone (7): A mixture of tetrahydrophenanthridone 6 (50.0 g, 0.206 mol), acetic anhydride (500 mL) and DMAP (1.0 g) is refluxed for 4 hours. Upon cooling a solid precipitates from solution. The precipitate is collected by filtration and sucked to dryness. The filtrate is evaporated under reduced pressure (40°), the resulting solid is triturated with ethanol, filtered, and the filter cake is combined with the one obtained previously. After an additional trituration of the combined product with ethanol, the solid is filtered off, washed with ice-cold ethanol and air dried. Yield: 40.0 g (62%); m.p. 155-157°, lit. 157-158°.

4H-r.3H-trans.3-(3',4'-Methylenedioxy-6'-carboxyphenyl)-4-acetamino-1-cyclohexene (8): N-acetylphenanthridone 7 (60.0 g, 0.2 mol) is suspended in methanol (1 L), then a solution of potassium hydroxide (22.4 g, 0.4 mol) in water (400 mL) is added, and the flask is immersed in a water bath preheated to 80°. The reaction mixture reaches a temperature of 70°C, and the reaction is kept at that temperature for 15 minutes. When cooled to room temperature a white solid starts to separate. The volume of the reaction mixture is reduced to 400 ml and the resulting precipitate is collected by filtration, washed with water (2 x 25 mL) and dried in air. It can be shown that the obtained precipitate is starting material 7.

The filtrate is cooled in an ice bath and acidified with con. hydrochloric acid to form a white precipitate that is filtered off, washed with water (2 x 25 mL) and dried in air. This product agrees in its spectral and analytical data with the structural assignment for 8, hence it is taken in the next step without further purification. Yield: 40.0 g (62%); m.p. 200-201°, lit. 198-201°. Recovered starting material: 20.5 g (37.0%).

4aH-r.1H-trans.10bH-cis.4H-trans.1-Acetylamino-4-bromo-8.9-methylene-dioxy-1.2.3.4.4a.10b-hexahydrobenzo[b,d]pyrone-6 (9): To a well-stirred suspension of acetylamino carboxylic acid 8 (145.3 g, 0.48 mol) in tetrahydrofuran (2 L) powdered N-bromosuccinimide (89.0 g, 0.497 mol) is added in a single portion. Most of the solid dissolves, and a crystalline material begins to separate. After stirring for 1 hour the reaction mixture is cooled in an ice bath, then the precipitate is collected by filtration, washed with cold tetrahydrofurane, and dried. Yield: 175.5 g (93.3%); m.p. 265-267°, lit. 270°.

4aH-r.1H-trans.10bH-cis.1-Acetylamino-8.9-methylenedioxy-1.2.4a.10b-tetrahydridibenzo[b,d]pyrone-6 (10): A mixture of acetylaminobromolactone 9 (456.1 g, 1.163 mol) and DBU (182 g, 1.2 mol) in pyridine (5.0 L) is kept at reflux for 8½ hours under anhydrous conditions. After cooling overnight a crystalline material precipitates. The solid is filtered off and slurried with water for 10 minutes, filtered, and air-dried. The filtrate is evaporated to dryness at oil vacuum pressure, and the solid residue is slurried in water for 10 minutes, filtered, air-dried, and combined with the previous precipitate. Yield: 339.0 g (93.7%); m.p. 270-272°, lit. 263-267°.

4aH-r.1H-trans.1-Hydroxy-8.9-methylenedioxy-1.4.4a.10b-tetrahydro-6(5H)phenanthridone (11): A mixture of acetylaminolactone 10 (60.0 g, 0.193 mol) and ethanol (150 mL) is treated with a solution of NaOH (30 g) in water (150 mL), then the reaction is heated to 90-95°C, and kept at that temperature for 8 hours. A small amount of water is added periodically to dissolve a solid that separates. The reaction mixture is allowed to cool to room temperature overnight, the precipitated solid is collected by filtration, washed with water and dried in air. The filtrate is acidified with concentrated hydrochloric acid, the resulting precipitate is filtered, washed with water (3 x 100 mL) and dried in air. Both precipitated solids are found to be identical therefore they are combined, however, NMR-analysis indicates the presence of substantial amounts of starting material. The product is redissolved in 20% sodium hydroxide (300 mL), kept at 120° for five hours, cooled, and reprecipitated. Repeating the procedure five times the obtained product does not show any more contamination. Yield: 94.0 g; m.p. 260-280° (dec.), lit. 265-280° (dec.).

4aH-r.1H-trans.1-(2'-Tetrahydropyranyloxy)-8.9-methylenedioxy-1.4.4a.10b-tetrahydro-6(5H)phenanthridone (12): To a suspension of hydroxyphenanthridone 11 (50 g, 0.193 mol) in dichloromethane (1.5 L) is added dihydropyran (70 mL, 0.857 mol) and p-toluene sulfonic acid (8.0 g). The reaction mixture is stirred for 72 hours with intermittent warming to 35°. The undissolved starting material is removed by filtration and the filtrate is washed with saturated sodium bicarbonate solution (2 x 500 mL) and water (500 mL). The organic layer is dried with sodium sulfate and evaporated to obtain a solid. Ethanol (25 mL) and ether (500 mL) are added to the solid and kept overnight at room temperature. The solid is filtered, washed with ether (2 x 200 mL) and air-dried to yield a white crystalline material. Yield: 54.0 g; m.p. 218°.

4aH-r.1H-trans.1-(2'-Tetrahydropyranyloxy)-2,3-epoxy-8,9-methylenedioxy-1,4,4a,10b-tetrahydro-6(5H)phenanthridone (13): Phenanthridone derivative **12** (82.0 g, 0.239 mol) and 3-chloroperoxybenzoic acid (82.0 g, 0.478 mol) are dissolved in methylene chloride (1.4 L) and stirred for two days. The organic solution is washed with saturated sodium bicarbonate solution (2 x 600 mL) and water (2 x 500 mL), dried over sodium sulfate and concentrated under reduced pressure to a volume of approx. 75 mL. Ether (200 mL) is added and the resulting precipitate is collected by filtration and washed with ether (100 mL). Yield: 71.1 g; m.p. 243°, lit. 250°.

4H-r.1H-trans.2H-cis.10bH-trans.1-(2'-Tetrahydropyranyloxy)-2-hydroxy-8,9-methylenedioxy-1,2,4a,10b-tetrahydro-6(5H)-phenanthridone (14): To a suspension of diphenyldiselenide (25.3 g, 0.081 mol) in anhydrous ethanol (500 mL) is added sodium borohydride (6.5 g, 0.17 mol) in small portions, while controlling the temperature with an ice bath. To the clear solution epoxide **13** (54.0 g, 0.15 mol) is added in one portion, then the reaction mixture is maintained at reflux temperature for two hours. Subsequently, the volume of the reaction solution is reduced to 250 mL by evaporation under reduced pressure, tetrahydrofuran (750 mL) is added, and the temperature is adjusted to about 3° in an ice bath. Hydrogen peroxide (30 wt. %, 250 mL, 2.2 mol equiv.) is slowly added while stirring, during which time a white solid precipitates. The reaction mixture is heated to reflux temperature, and reflux is maintained for 7 hours during which time all the solid dissolves and the reaction mixture becomes dark in color. Upon cooling, water (2 L) is added and the mixture is extracted with ethyl acetate (3 x 700 mL). The organic phase is washed with water (2 x 500 mL), dried over sodium sulfate, and upon evaporation of the solvent a white solid is obtained, which is washed with ether (2 x 200 mL). Yield: 38.5 g; m.p. 230-235°; lit. 232°.

The product (7 g) is recrystallized from ethanol to give 6.7 g pure product.

4aH-r.1H-trans.2H-cis.3H-trans.4H-trans.10bH-trans-1-(2'-Tetrahydropyranyloxy)-2,3,4-trihydroxy-8,9-methylenedioxy-1,2,3,4,4a,10b-hexahydro-6(5H)phenanthridone (15): Osmium tetroxide (100 mg) is added to N-Methylmorpholine-N-oxide (5.5 g, 47 mmol), dissolved in a mixture of t-butanol (30 mL), acetone (30 mL) and water (20 mL). A solution of intermediate **14** (10.0 g, 28 mmol) in t-butanol (500 mL) and acetone (200 mL) is added to the osmium tetroxide solution and the resulting mixture is stirred for 24 hours, when additional N-methylmorpholine-N-oxide (4 g, 34 mmol) and osmium tetroxide (100 g) is added. The reaction is completed after stirring an additional 24 hours, and the solvent is removed under reduced pressure. The residual solvent is eliminated by co-evaporation with ethanol (50 mL twice). Upon trituration of the residue with ethanol (30 mL) the product crystallizes and it is collected by filtration. Yield: 8.1 g (74%); m.p. 218° (decomp).

AVS 360 TA

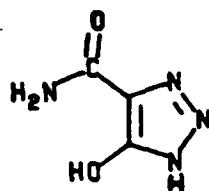
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V. DISCUSSION OF UNCOMPLETED TARGET COMPOUNDS

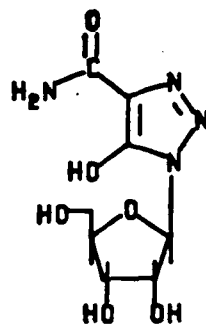
List of Compounds in Progress:

AVS 94	5-Hydroxy-1,2,3-triazole-4-carboxamide
AVS 136	5-Hydroxy-1- β -D-ribofuranosyl-1,2,3-triazole-4-carboxamide
AVS 360	Lycoricidine triacetate
AVS TFN	Protected and unprotected Thiazofurin nitrile
	Ribavirin Metabolites
	Prodrug Ester
AVS 206	Ribavirin amidine hydrochloride

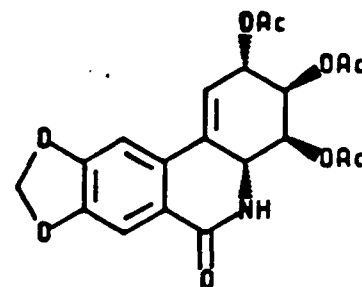
Structures of Compounds in Progress



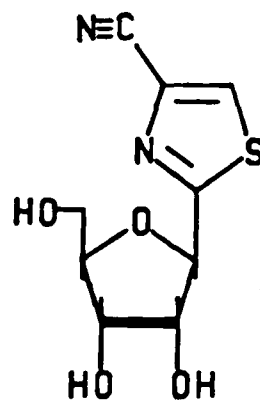
AVS94



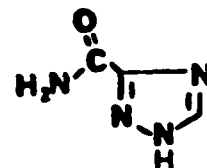
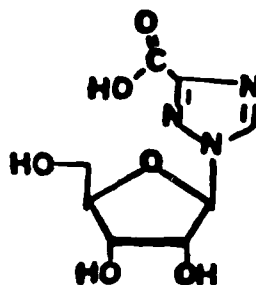
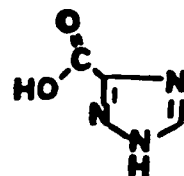
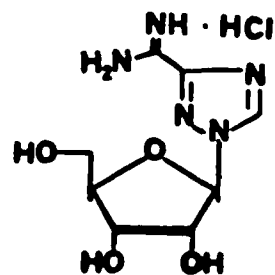
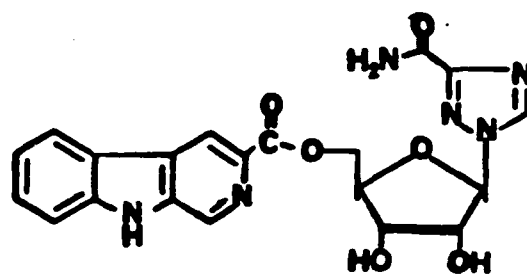
AVS136



AVS360

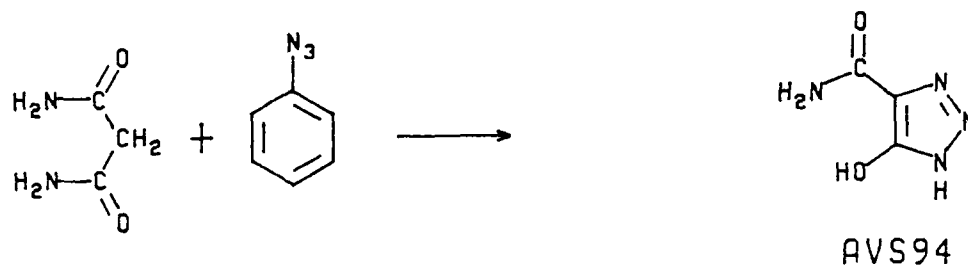


AVS-TFN



Q. 5-Hydroxy-1,2,3-triazole-4-carboxamide, AVS 94

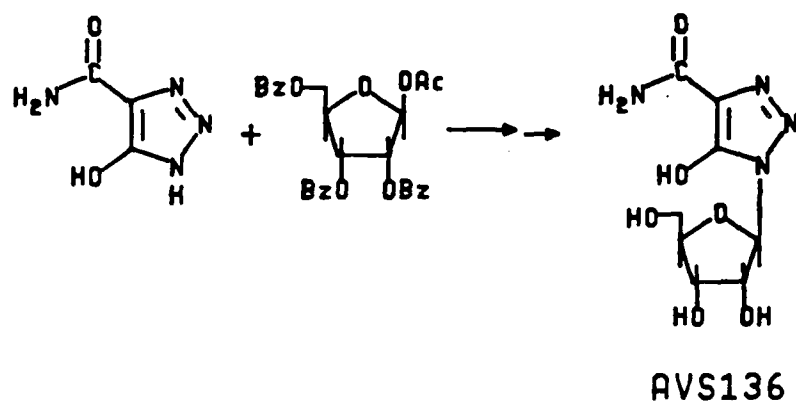
AVS 94 will be synthesized according to the following scheme¹².



Presently the starting materials are being prepared to initiate the synthesis of AVS 94.

R. 5-Hydroxy-1- β -D-ribofuranosyl-1,2,3-triazole-4-carboxamide. AVS 136

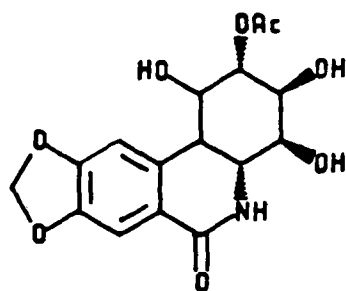
AVS 136 will be synthesized according to the following scheme¹⁸.



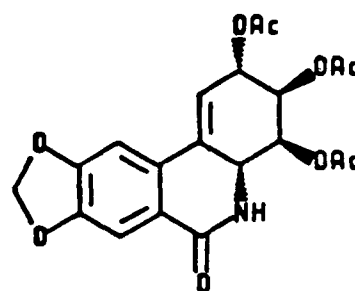
Presently the starting materials are being collected to initiate the synthesis of AVS 136.

S. Lycoricidine triacetate, AVS 360¹⁰

A final preparation of Lycoricidine triacetate is being prepared, using up all the intermediates left over from a preparation which was submitted last year.



AVS360MA

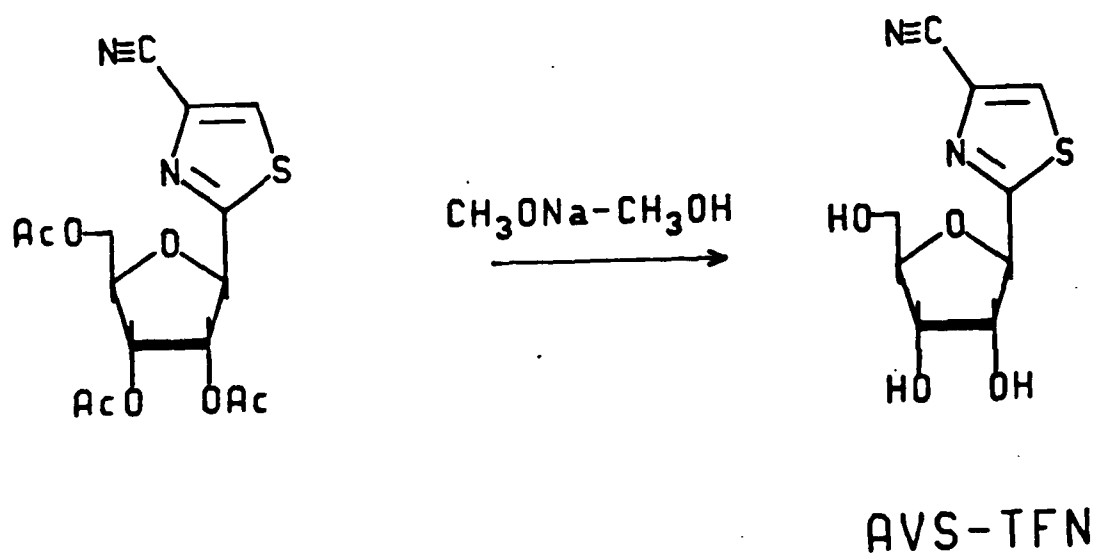


AVS360

About 3 to 4 grams of AVS 360 can be expected to be shipped soon.

T. Protected and unprotected Thiazofurin nitrile. AVS TFN

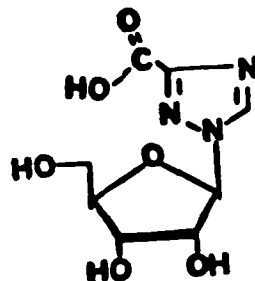
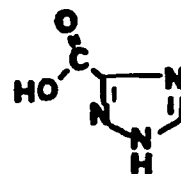
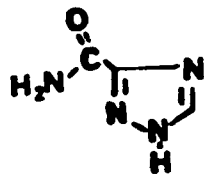
Triacetyl thiazofurin (50 g) has been dehydrated to form the carbonitrile.



A portion of the acetylcyanitrile has been deprotected, and the blocked and deblocked thiazofurin nitriles will be submitted in the near future.

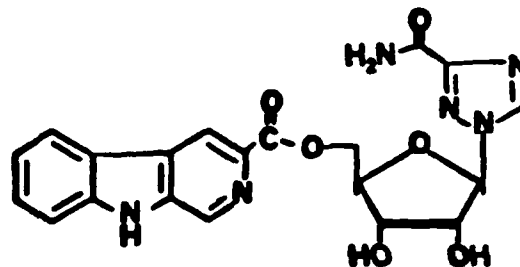
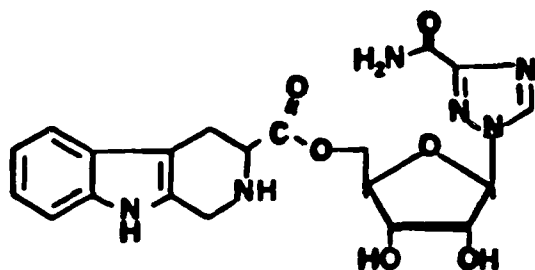
U. Ribavirin Metabolites

The following three compounds, 1,2,4-triazole-3-carboxamide (1), 1,2,4-triazole-3-carboxylic acid (2), and 1- β -D-ribofuranosyl-1,2,4-triazole-3-carboxylic acid (3), found to be metabolic intermediates of ribavirin are presently being prepared, and their submission can be expected shortly.



V. Prodrug Ester¹⁴

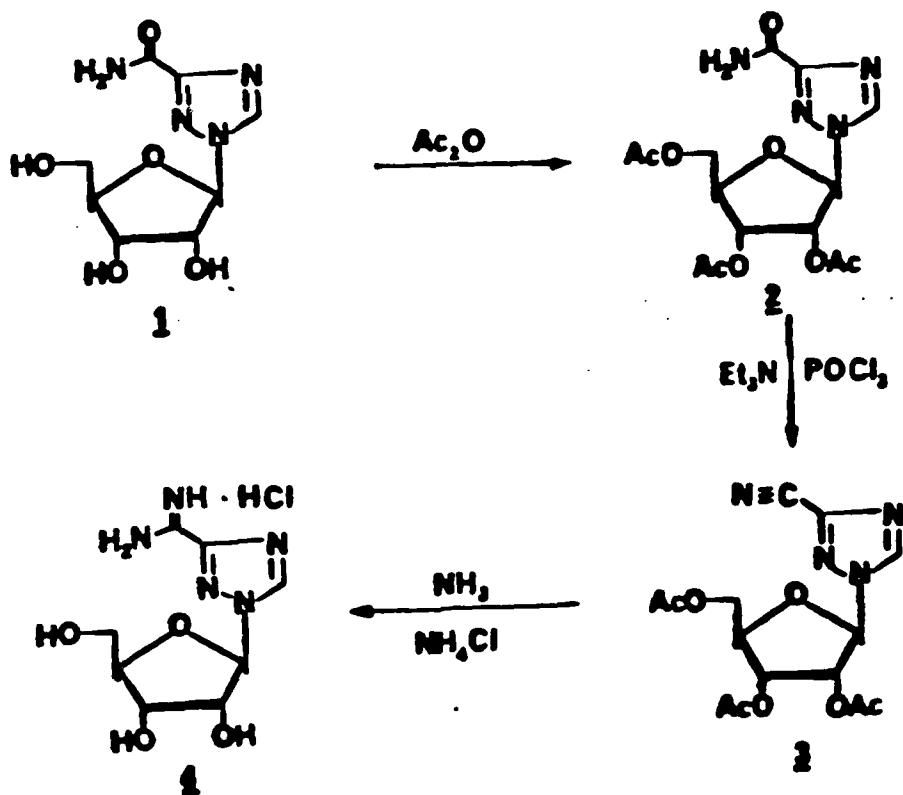
It has been proposed to couple β -carbolines with ribavirin to possibly form a new prodrug. Presently the synthetic steps are being investigated to optimize reaction conditions.



Recently not much time had been allocated to pursue this project.

W. Ribavirin amidine hydrochloride, AVS 206^{1,4}

Recently the preparation of 2 Kg of AVS 206 has been assigned to this contract. This large-scale preparation necessitates the synthesis of about 6 Kg of ribavirin, which will be performed according to previously reported methods from this laboratory. The transformation of ribavirin to AVS 206 will be accomplished according to the following scheme.



At the moment suitable suppliers are being screened and questioned to allow for an expedient and cost-effective purchase of the required starting materials.

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VII. ACKNOWLEDGMENTS

The personnel assigned to this contract during the past annual period were: Ernst M. Schubert, Ph.D., Principal Investigator; Krishna Upadhyaya, Ph.D., Principal Assistant, and Jay DaRe, B.S., Chemist.

Report Submitted By:
Pharm-Eco Laboratories, Inc.

A handwritten signature in cursive script, appearing to read "Ernst Schubert", is written over a horizontal line.

Ernst M. Schubert, Ph.D.
Principal Investigator
(805) 527-3630

VIII. APPENDIX

Reprint of Publication

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August

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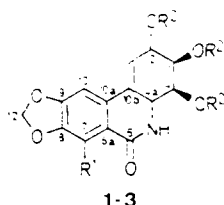
Improved Synthesis of Lycoricidine Triacetate

Bheemarao G. Ugarkar, Jay DaRe, Ernst M. Schubert*

Pharm-Eco Laboratories, Inc., 2355 Chain Drive, Simi Valley, CA 93065, U.S.A.

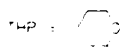
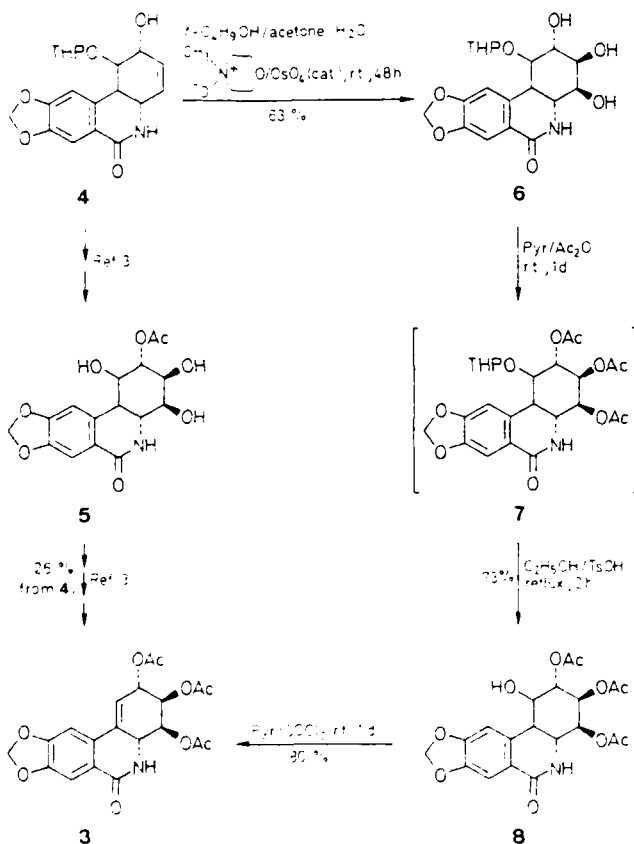
The synthesis of lycoricidine triacetate by a modified pathway is described. In this preparation, catalytic amounts of osmium tetroxide are used to stereospecifically introduce two hydroxy groups, rendering the title compound via two novel intermediates.

Lycoricidine (1) and lycoricidinol (2), two constituents found in *Amaryllidaceae* plants, show strong growth-inhibiting action in the rice seedling test, and they exhibit anti-tumor activity against Ehrlich carcinoma.¹ Such antimitotic behavior prompted further investigations to establish their configurations and conformations,¹ which were subsequently confirmed by total synthesis.^{2,3}



	R ¹	R ²
1	H	H
2	OH	H
3	H	Ac

During recent *in vitro* testing of lycoricidine triacetate (3) it displayed antiviral activity,⁴ and a proposed *in vivo* study necessitated the preparation of a larger quantity of 3. The isolation of lycoricidine from plant material is impractical since



its abundance in fresh bulbs of *Lycoris* is $<0.00032\%$, while a reported synthesis produced lycoricidine in a 21-step procedure with 1.5% overall yield, starting with piperonal. After establishing the desired phenanthridone intermediate 4 following a twelve-step procedure, acetylation and removal of the tetrahydropyranyl group is followed by stereospecific *cis*-hydroxylation with equimolar amounts of osmium(VIII) oxide to yield intermediate 5.

The two *cis*-hydroxy groups in 5 are protected as the isopropylidene acetal to allow for the introduction of a double bond between C-1 and C-10b by dehydration. Acetylation, following removal of the isopropylidene group, renders lycoricidine triacetate in 26% yield, based on intermediate 4.

The improved preparation of 3, as shown in the scheme, starts with the direct *cis*-hydroxylation of 4 by utilizing only catalytic amounts of osmium(VIII) oxide, according to a method described in Lit.⁵ to yield trihydroxy compound 6. The absence of isomers in the oxidation mixture prior to work-up, as indicated by TLC, attests to the applicability of osmium(VIII) oxide as a highly stereoselective reagent.

After acetylation of the three hydroxy groups in 6, the resulting *O*-tetrahydropyranyl derivative 7 is hydrolyzed without prior purification to render intermediate 8, which upon dehydration yields lycoricidine triacetate in 37% overall yield, starting with 4.

Spectral and analytical data confirm the structural assignments of 3 and, based on 400 MHz ¹H-NMR, ¹³C-NMR, COSY, and HOMCOR spectra, the spatial arrangement at the four chiral carbons is in agreement with the absolute structure assigned to lycoricidine. Two different melting points have been reported for lycoricidine triacetate, depending on its origin,³ but such a difference could be a result of the mode of recrystallization to obtain a product of differing crystal structure. Hydrolysis of the prepared lycoricidine triacetate, according to Lit.³, produces lycoricidine (1), whose spectral and analytical data agree with the reported values for lycoricidine of natural origin.

Presently, lycoricidine triacetate (3) is being screened for its *in vivo* antiviral activity, and if proven useful as an antiviral agent, it can readily be prepared according to the reported modifications. They make the procedure economically more attractive for scale-up work because they reduce the number of required steps, thus improving the overall yield, while significantly reducing the hazards of handling and disposing of large amounts of highly toxic osmium oxides.⁶

All solvents used were of reagent grade. Melting points were taken on a Thomas-Hoover capillary melting point apparatus and are uncorrected. TLC was run on Silica Gel GF plates (Analtech, Newark, Del.) where the products were visualized by UV-absorbance, or by iodine stains. Microanalyses were performed by MHW Laboratories, Phoenix, Arizona. Mass spectral analyses were taken on a Varian Model 311A spectrometer by Dr. K. H. Schram, College of Pharmacy, University of Arizona, Tucson, AZ. IR spectra were obtained on a Beckman AccuLab 2, and UV spectra were recorded on a Beckman Model 25 spectrophotometer. In addition to the standard NMR spectra, taken on a Varian EM 390 spectrometer, 400 MHz ¹H-NMR, ¹³C-NMR, attached proton test (APT), homonuclear correlation (COSY), and heteronuclear correlation (HETCOR) spectra were recorded by SRI International, Menlo Park, California.

4aH-1H-*trans*,2H-*cis*,3H-*trans*,4H-*trans*,10bH-*trans*-1-(2-Tetrahydropyranyloxy)-2,3,4-trihydroxy-8,9-methylenedioxy-1,2,3,4,4a,10b-hexahydro-6(5H)phenanthridone (6):

To a solution of *N*-methylmorpholine *N*-oxide⁷ (16.0 g, 0.137 mol) in *t*-BuOH (50 mL), acetone (50 mL), and H₂O (20 mL), osmium tetroxide

(260 mg, 1 mmol) is added. To this reaction medium is added a solution of phenanthridone derivative 4² (28.8 g, 80 mmol) in *t*-BuOH (700 mL) over a 10 min period, followed by continued stirring for 48 h. Decolorizing carbon is added to the dark solution, and after stirring for 3 h at room temperature the mixture is filtered through a Celite bed. The resulting pale-yellow solution is evaporated under reduced pressure. The remaining oil is triturated with EtOH (25 mL), and the resulting crystalline material is collected by filtration. The solid is suspended in H₂O (100 mL). This suspension is stirred for 1 h, and filtered. The off-white solid is washed with H₂O, and air-dried to give trihydroxy compound 6; yield: 20.0 g (63%); m.p. 222–223°C; TLC (CHCl₃/MeOH 6:1), R_f: 0.60.

C₁₉H₂₃NO₈ calc. C 58.01 H 5.89 N 3.56
(393.4) found 58.42 5.96 3.62

IR (KBr): ν = 3600–3200 (br), 2970, 1665, 1610, 1495, 1460, 1390, 1355, 1260, 1075, 1035 cm⁻¹.

¹H-NMR (DMSO-*d*₆/TMS): δ = 1.4 (br.s, 9H, tetrahydropyran); 3.1–4.5 (m, 8H, 5H_{aliph} + 3OH); 4.9 (s, 1H, H-4a); 6.15 (D, 2H, *J* = 2.5 Hz, –CH₂–); 6.8 (s, 1H, NH); 7.05 (s, 1H, H-10); 7.4 (s, 1H, H-7).

4aH-*v*,1H-*trans*,2H-*cis*,3H-*trans*,4H-*trans*,10bH-*trans*-1-Hydroxy-2,3,4-triacetoxy-8,9-methylenedioxy-1,2,3,4,4a,10b-hexahydro-6(5H)-phenanthridone (8):

A mixture of trihydroxy compound 6 (18.6 g, 0.047 mol), pyridine (200 mL), and acetic anhydride (200 mL) is stirred at room temperature overnight. Acetic anhydride and pyridine are evaporated under reduced pressure, followed by evaporation with EtOH to remove traces of pyridine. The residual material is soaked in EtOH (50 mL) and chilled. The resulting crystalline material is collected by filtration, washed with EtOH (50 mL), and air-dried; yield of intermediate 7: 22.0 g; m.p. 275°C.

Without further characterization, intermediate 7 is suspended in EtOH (500 mL), *p*-toluenesulfonic acid (500 g) is added, and the mixture is kept at reflux for 2 h. Upon cooling, the crystalline material is filtered, washed with cold EtOH (2 × 25 mL), and dried in air; yield of 8: 15.0 g (73%); m.p. 303–304°C; TLC (CHCl₃/MeOH 6:1), R_f: 0.75.

C₂₀H₂₁NO₁₆ calc. C 55.17 H 4.86 N 3.21
435.4 found 54.95 4.90 3.13

IR (KBr): ν = 3460, 3180, 3070, 2965, 1740, 1670, 1480, 1450, 1360, 1250, 1215, 1040, 920 cm⁻¹.

¹H-NMR (DMSO-*d*₆/TMS): δ = 2.05 (s, 6H, 2 acetyl); 2.15 (s, 3H, 1 acetyl); 3.1–5.5 (m, 7H_{aliph}); 6.15 (s, 2H, –CH₂–); 6.9 (s, 1H, NH); 7.35 (s, 1H, H-10) 7.9 (s, 1H, H-7).

Lycoricidine Triacetate [4aH-*v*,1H-*trans*,2H-*cis*,3H-*trans*,4H-*trans*,10bH-*trans*,2,3,4-Triacetoxy-8,9-methylenedioxy-2,3,4,4a-tetrahydro-6(5H)-phenanthridone] (3):

Intermediate 8 (7.0 g, 18 mmol) is dissolved in pyridine (70 mL) while stirring; upon cooling of the mixture, thionyl chloride (10 mL) is added over a 20 min period. Stirring is continued overnight while the mixture is allowed to warm up to room temperature. CH₂Cl₂ (500 mL) is added, and the solution is washed with H₂O (2 × 500 mL), with 10% hydrochloric acid (2 × 250 mL), and H₂O (2 × 500 mL). The organic phase is dried (Na₂SO₄) and evaporated under reduced pressure to yield a white solid. Upon trituration with MeOH (40 mL), the crystalline material is filtered, washed with MeOH (2 × 20 mL) and dried in air. This product is recrystallized from CH₂Cl₂/MeOH, 1:1 (50 mL) to give the pure product 3. From the mother liquor, a second crop is obtained; total yield: 6.0 g (80%); m.p. 266–268° (dec.).

C₂₀H₁₉NO₉ calc. C 57.55 H 4.59 N 3.35
417.3 found 57.46 4.50 3.36

The MS-, IR-, and UV-spectral data all agree with the reported values.³

¹H-NMR (CDCl₃/TMS): δ = 2.09 (s, 3H, acetyl); 2.11 (s, 3H, acetyl); 2.15 (s, 3H, acetyl); 4.65 (d, 1H, *J* = 10 Hz, H-4a); 5.2–5.4 (m, 3H, H-2, H-3, H-4); 6.10 (s, 3H, H-1, H-12); 7.00 (s, 1H, H-10); 7.35 (s, 1H, NH); 7.50 (s, 1H, H-7).

¹³C-NMR (CDCl₃/TMS): δ = 21.0 (3C, acetyl), 50.1 (C-4a), 68.2 (C-4), 68.6 (C-3), 71.2 (C-2), 102.0 (C-12), 103.4 (C-10), 107.5 (C-7), 117.0 (C-1), 122.5 (C-10b), 130.3 (C-10a), 134.1 (C-6a), 149.2 (C-9), 151.7 (C-8), 164.3 (C-6), 169.5 (C=O), 169.7 (C=O), 170.4 (C=O).

This study was performed under Contract #DAMD17-85-C-5071, U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, Maryland. The findings in this communication are those of the authors and should not be construed as an official Department of the Army position, unless so designated by other documentation.

Received: 22 November 1986; revised: 25 February 1987

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- (7) Purchased from Aldrich Chemical Co., Milwaukee, Wisconsin.

Preparation of Dimethyl (1-Formylalkyl)phosphonates via Singlet Oxygen Adducts

Mitsuji Yamashita,* Hiroyuki Nomoto, Hiroyuki Imoto

Department of Applied Chemistry, Faculty of Engineering, Shizuoka University, Hamamatsu 432, Japan

The nitro group of dimethyl (1-nitromethylalkyl)phosphonates is conveniently converted into a formyl group by reaction with singlet oxygen to give dimethyl (1-formylalkyl)phosphonates. (1-Formylbutyl)diphenylphosphine oxide is prepared by the same reaction.

The conversion of nitroalkanes into carbonyl compounds is a synthetically useful functional group interconversion. The reaction is usually carried out under strongly acidic,¹ basic oxidative,^{2–8} neutral oxidative,⁹ or neutral reductive conditions.^{7,10–12} Surprisingly, our numerous attempts to effect the analogous conversion of organophosphorus compounds having a *P*-(2-nitroalkyl) group under various conditions were unsuccessful. However, we then found that oxidation with ozone conveniently converts [1-(nitromethyl)alkyl]diphenylphosphine oxides into (1-formylalkyl)diphenylphosphine oxides;¹³ however, overoxidation is problematic and does not allow for efficient scale-up.

Phosphoric esters are widely known to exert a great variety of vital functions. Some phosphonates having a C-P bond, formally being prepared by replacing the O-atom of a P-O-C linkage of phosphoric esters by the isoelectronic CH₂ group, are also naturally occurring, and their metabolism has been investigated.¹⁴ Phosphonic acid derivatives such as (–)-(1*R*, 2*S*)-1,2-epoxypropylphosphonic acid (phosphonomycin) and dimethyl 1-hydroxy-2,2,2-trichloroethylphosphonate (Dipterex) are active substances as antibiotics and pesticides^{15,16} and are therefore of practical importance.

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